This is to certify that the

dissertation entitled

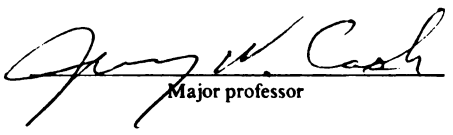
Postharvest Treatment to Reduce or Remove Ethylenebisdithiocarbamate (EBDC) Fungicides from Apples and Apple Products & Elucidation of Possible Degradation By-products and Pathways

presented by

Eun-Sun Hwang

has been accepted towards fulfillment of the requirements for

Ph.D. degree in Food Science/ Environmental Toxicology



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POSTHARVEST TREATMENT TO REDUCE OR REMOVE ETHYLENEBISDITHIOCARBAMATE (EBDC) FUNGICIDE RESIDUES FROM APPLES & APPLE PRODUCTS AND ELUCIDATION OF POSSIBLE DEGRADATION BY-PRODUCTS & PATHWAYS

By

Eun-Sun Hwang

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science & Human Nutrition
Institute for Environmental Toxicology

1999

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ABSTRACT

POSTHARVEST TREATMENT TO REDUCE OR REMOVE ETHYLENEBISDITHIOCARBAMATE (EBDC) FUNGICIDE RESIDUES FROM APPLES AND APPLE PRODUCTS & ELUCIDATION OF POSSIBLE DEGRADATION BY-PRODUCTS AND PATHWAYS

By

Eun-Sun Hwang

The overall goal of this research was to reduce or eliminate mancozeb residues in apples and apple products, determine the effectiveness of different postharvest treatments and processing on the reduction of mancozeb and ethylenethiourea (ETU) residues and elucidate possible degradation products and pathways of this pesticide when treated with various oxidation agents.

In the first part of the research, laboratory studies were conducted using a model system to determine the effects of calcium hypochlorite (50, 250 & 500 ppm), chlorine dioxide (5 & 10 ppm), ozone (1 & 3 ppm) and hydrogen peroxyacetic acid (HPAA) (5 & 50 ppm) at pH 4.6, 7.0, 10.7 and at 10°C and 21°C on the degradation of mancozeb in solution over a 30 minute period. Rate of mancozeb degradation was dependent on pH, with pH 7.0 being the most effective. Under controlled conditions, ETU residue concentrations increased up to 15 minutes reaction time and then decreased in all three pH ranges. Ozonation was

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effective in the degradation of ETU residue in mancozeb solution. Chlorine dioxide was an excellent degradation agent at low concentration.

The second part of this study included laboratory whole fruit studies. Mancozeb was spiked on the surface of apples at two different concentrations and the effectiveness of each oxidizing agent was determined on the reduction and degradation of mancozeb and ETU residues on actual fruit as compared to the solution experiments. The results showed similar patterns to the model system studies.

In the third part of this study, mancozeb was applied on orchard apples throughout the growing season at the recommended rate. Postharvest wash treatments were used, based on results of the model system study: (1) no wash, (2) water wash, (3) calcium hypochlorite wash @ 50 and 500 ppm (4) chlorine dioxide wash @ 10 ppm (5) ozone wash @ 3 ppm and (6) HPAA wash @ 50 ppm. Wash treated apples were processed as whole fruits, slices, sauce (peeled and unpeeled), juice and pomace and frozen at –20°C until residue analysis. When wash treatments were combined with processing, mancozeb and ETU were reduced by 100% (i.e., below detectable limits).

The last part of this study involved investigation of degradation products and possible pathways during chemical oxidation reaction. Samples were detected by Time–of–Flight Mass Spectrometry (TOFMS) with an electron ionization source. Several degradation by-products were detected and identified.

I dedi

For my paren

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I dedicate this dissertation to my beloved parents and God.

For my parents, whose unspoken love, expectations and understanding have always been treasured by their daughter.

I thank God for my very good fortune for I am living a very blessed life indeed.

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I wish to express my sincere gratitude to all those people who have in one way or another contributed to the successful completion of my study. I have been blessed to meet numerous people with the knowledge and patience necessary to aid me in my experience. In particular, I would like to express very special thanks to my major advisor, Dr. Jerry N. Cash, for his kind support, endless understanding, warm encouragement and professional guidance. His support and trust in my ability to learn gave me the determination to complete my Ph.D. program and this dissertation.

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I owe a great debt of thanks to my teachers, colleague and friends who freely gave me a tremendous amount of technical and personal support prior to and throughout this study. There are many people who assisted, but unfortunately, they can not all be mentioned by name. Of these people, I wish to give thanks to my lab members, Muhammad Siddiq, Chris Vandervoort, Violet Morre and other fellow graduate students for their help and friendship.

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LITERATURE REVIEW

A. General Aspects of Pesticides

Federal law defines a pesticide as "any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest" (CFR, 1988). Pesticides may also be described as any physical, chemical or biological agent that will kill an undesirable plant or animal pest (Ecobichon, 1996). Pesticide is a general term for many types of products including insecticides, herbicides, fungicides and rodenticides. Pesticides may be chemical or bacterial, natural or man-made. There are approximately 320 active pesticide ingredients that are available in several thousand different registered formulations (Hotchkiss, 1992). The U.S. Environmental Protection Agency (EPA) reported over 811 million pounds of pesticides, excluding wood preservatives and disinfectants, used in U.S. agriculture in 1993, at a cost of $6.1 million (Schubert *et al.*, 1996).

Pesticide use in agriculture over the last several decades has proven to be a great benefit to the production of food. Pesticides protect crops by controlling insects, diseases, weeds, fungi (mold) and other pests. They work because they are toxic to target organisms or otherwise

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disrupt natural processes necessary for the organisms' survival. Pesticide use has improved both the efficiency of growing crops and the quality of food produced. Protecting crops from pests gives higher yields and better quality, resulting in greater variety and availability of food at a low cost. However, along with the benefits, there are the potential effects of trace amounts of pesticide residues remaining on some commodities at the time of harvest or sale to the general public. Pesticides are potentially harmful to humans and can cause various health problems such as cancer, birth defects, changes in genetic material that may be inherited by the next generation (genetic mutations), and nerve damage, among other debilitating or lethal effects.

Pesticides are applied directly to many crops, especially fresh fruit and vegetables. Many factors can influence the nature and extent of pesticide residues on a crop, such as sunlight, water, bacteria in the soils and other physical factors. The resulting breakdown products may be biologically inactive compounds or may be chemicals that are themselves toxic (Cooley and Manning, 1995).

Figure 1 shows the pesticide use on major crops between 1964 and 1997. Pesticide use increased from 1964 to 1982 but decreased from 1982 through 1991. This is probably due to integrated pest management (IPM) practices designed to maintain disease and pest control using minimum levels of pesticide. After 1991, the overall pesticide use

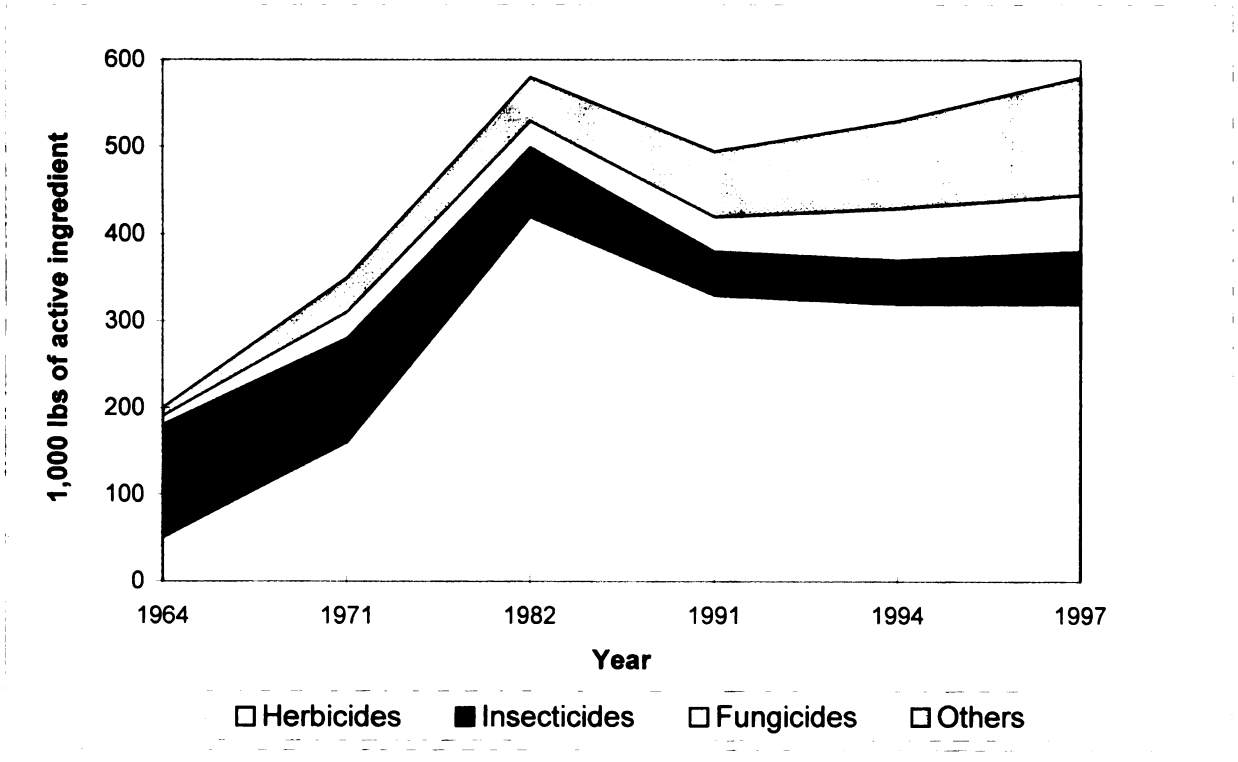

Figure 1. Pesticide use of major crops, 1964-1997 (source: USDA, ERS).

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increased slightly. This indicates that consumers still demand good sensory quality of products even though they have concerns about pesticide residues. Food safety has received increased attention in recent years as a major consumer concern. In several consumer surveys, 70–80% of the respondents expressed concern about the health risks associated with pesticide residues (Food Marketing Institute, 1992; Ott *et al.*, 1991). This has resulted in extensive research on the biological efficacy and environmental fate of pesticides.

B. The Fate of Pesticides in the Environment after Application

Pesticides can be introduced directly into the environment in a liquid phase, as a dispersion or solution, or in the solid phase, as a powder, dust, microcapsule, or granule. The pesticides are exposed to many agents capable of transforming them into various other forms. After entering both target and non-target biota, pesticides are subjected to attack by detoxification enzymes. However, the major proportion of an applied pesticide does not immediately enter any organism, but remains in soil, water or air where it is subjected to further transformation and transport to different locations, as well as, uptake by organisms at that site (Fuhr, 1982). Figure 2 is a simplified scheme illustrating the various processes to which pesticides applied for plant protection are subjected (direct application to soil and/or to plant surface is the main route of

Figure 2.

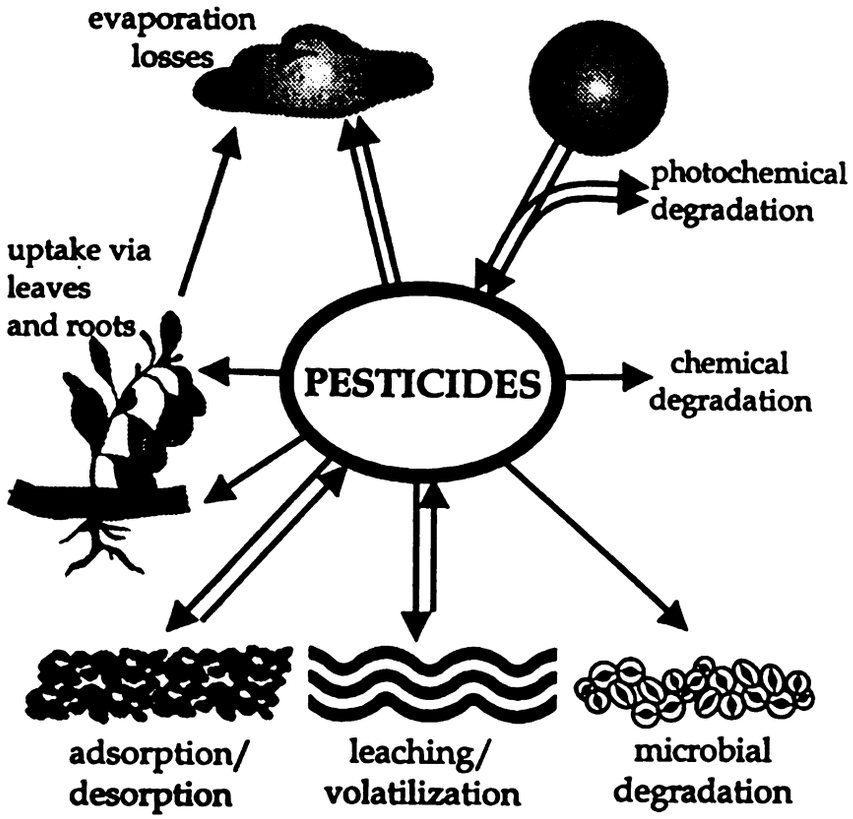

Figure 2. The fate of pesticide in the environment after application (Schubert et al., 1996).

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pesticide input to the environment). The fate of a pesticide in the environment is governed by the retention, transformation, transport processes and the interaction of these processes. Retention is the consequence of interaction between the pesticide chemical and the soil particle surface or soil components. The retention processes are frequently described as adsorption or simply as sorption. Degradation tends to decrease the chemical's toxicity although occasionally the metabolic products could be even more toxic than the parent compound. Volatilization leads to the distribution of pesticides from the soil to the atmosphere. Leaching leads to the movement of the pesticides toward the ground waters and overland flows move the pesticides into surface waters.

The air, water and soil in rural farming areas may be contaminated with pesticides or their degradation products. Pesticides also contaminate ecosystems and may produce harmful effects in wildlife. At the same time, the vast majority of adverse effects due to pesticides are largely unknown. Pesticide products to which we are exposed are a combination of chemical ingredients that include the active ingredients disclosed on the product label, which attack the target pest, and "inert" ingredients. However, the active ingredients are usually the smallest percentage of total ingredients, which are principally the undisclosed or secret "inert" ingredients. This part of the formulation can

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be biologically and chemically active and even more toxic than the actives, but are protected as trade secrets (Schubert *et al.*, 1996). Beyond these components of a formulation, a pesticide product also contains contaminants and breakdown products or metabolites. These, too, can be the most toxic part of the pesticide product (Schubert *et al.*, 1996).

Of all forms of pesticide pollution, groundwater degradation is especially serious, because groundwater is the source of public drinking water. Once groundwater contamination is discovered, clean–up is often neither technically nor economically feasible. The contamination of groundwater by pesticides is quite extensive. In a 1988 report, EPA documented the presence of 74 different pesticides in the groundwater of 32 states. In particular, EPA discovered widespread contamination by the pesticides aldicarb, atrazine and alachlor. A more extensive EPA study released in November 1990 found further evidence of contamination. Based on sampling results, EPA estimated that 10.4 percent of community water system wells and 4.2 percent of rural domestic wells in the U.S. contaminated at least one pesticide or pesticide degradation product. EPA's survey reveals that at a minimum, over 1.3 million people are drinking water contaminated with one or more pesticide from private wells.

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C. General Aspects of Fungicides

Fungicides have the longest history of the three main groups of crop protection agents (insecticides, herbicides and fungicides) (Uesugi, 1998). They are derived from a variety of structures ranging from simple inorganic compounds, such as sulfur and copper sulfate, through the aryl- and alkyl-mercurial compounds and chlorinated phenols to metal-containing derivatives of thiocarbamic acid (Ecobichon, 1996). Fungicides may be described as protective, curative or eradicative according to their mode of action. Protective fungicides, applied to the plant before the appearance of any phytopathic fungi, prevent infection by either sporicidal activity or by changing the physiological environment on the leaf surface. Curative fungicides are used when an infestation has already begun to invade the plant, and these chemicals function by penetrating the plant cuticle and destroying the young fungal mycelium growing in the epidermis of the plant, preventing further development. Eradicative fungicides control fungal development following the appearance of symptoms, usually after sporulation, by killing both the new spores and the mycelium and by penetrating the cuticle of the plant to the subdermal level (Kramer, 1983).

To be an effective fungicide, a chemical must posses the following properties: (1) low toxicity to the plant but high toxicity to the particular fungus; (2) active or capable of conversion (by plant or fungal

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enzymes) into a toxic intermediate; (3) the ability to penetrate fungal spores or the developing mycelium to reach a site of action; and (4) forms a protective, tenacious deposit on the plant surface that will be resistant to weathering by sunlight, rain and wind (Cremlyn, 1978). This list of properties is never fulfilled entirely by any single fungicides and all commercially available compounds show some phytotoxicity, lack of persistence due to environmental degradation and so forth. Thus, the timing of the application is critical in terms of the development of the plant as well as the fungus.

The topic of fungicidal toxicity has been extensively reviewed by Hayes (1982) and Edwards *et al.* (1991). With a few exceptions, most of these chemicals have a low toxicity to mammals. However, all fungicides are cytotoxic and most produce positive results in the usual in vitro microbial mutagenicity test systems. Public concern has been focused on the positive mutagenicity test obtained with many fungicides and the predictive possibility of both teratogenic and carcinogenic potential.

D. EBDC Fungicides

Ethylene bisdithiocarbamates (EBDCs) are one of the oldest and most widely used classes of organic fungicides in the world. They were first introduced during the 1940s and are widely used nonsystemic fungicides with low water solubility, which results in the pesticide

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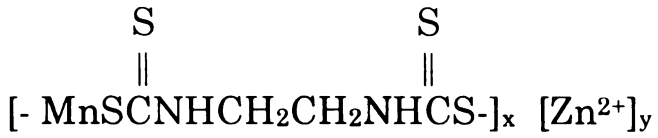
remaining as superficial deposits on the surface of treated crops. This allows it to be partly removed by water, especially on non-waxy crops such as strawberries (Federal Register, 1989). EBDCs have been used to control some 400 pathogens on more than 70 crops worldwide and approximately one-third of all fruits and vegetables in the United States are treated with EBDCs (Banrc, 1987). The major crops are apples, tomatoes, potatoes, grapes, bananas, corn and wheat. (EPA, 1989). The EBDCs registered for food uses in the U.S. are mancozeb, maneb, metiram, nabam and zineb (Lentza–Rizos, 1990). Figure 3 shows chemical structures of major EBDCs. These organic fungicides are usually more effective than inorganic fungicides because organic molecules tend to be more compatible with fungal cells which are surrounded by walls and membranes in which a lipid layer is important in exchanging substances through the layer. EBDCs act on various sites in fungal physiolgy. These types of multiple-site inhibiting fungicides, which are also called multisite inhibitors, are liable to act on organisms other than their targets. EBDCs are applied as their manganese and zinc complex form (maneb or mancozeb). The solubility, activity, and stability of the EBDCs are dependent of the metal ion form (Lentza–Rizos, 1990).

EBDCs fit well into integrated pest management (IPM) practices designed to maintain disease and pest control using minimum levels of pesticide. One of the most important assets of EBDC fungicides is that,



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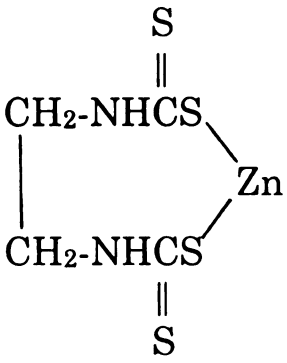

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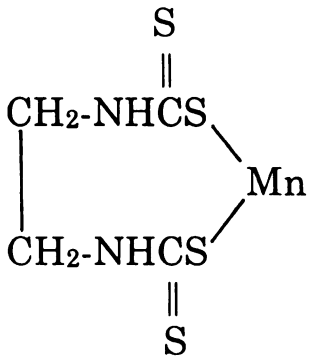

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Figure 3. Chemical structures of EBDCs.

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in all their years of use, no known disease resistance to them has developed, as is the case with many systemic fungicides (DuPont, 1992). Because EBDCs act in a preventive mode, the pathogen does not have the opportunity to infect the crop. EBDCs are also valuable in IPM programs because they are not harmful to beneficial insects. This helps reduce use of potentially more toxic pesticides. EBDCs are contact fungicides, which remain on the surface of the plant. A synergistic effect occurs when EBDCs are used with copper (DuPont, 1992).

Mancozeb (Dithane 75 DF®) is registered as a general use pesticide by the U.S. Environmental Protection Agency (EPA). It is a polymeric complex of ethylene bisdithiocarbamate manganese and zinc salt. It contains 75% of ethylene bisdithiocarbamate in which the ingredients are 15% of manganese, 1.87% of zinc and 58.13% of ethylene bisdithiocarbamate ion ($C_4H_6N_2S_4$) and 25.00% of inert ingredients. It is one of the most widely used EBDC fungicides to protect many fruits, vegetables, nuts and field crops against a wide spectrum of diseases, including potato blight, leaf spot, scab on apples and pears and rust on roses (DuPont, 1992). It is also used for seed treatment of cotton, potatoes, corn, safflower, sorghum, peanuts, tomatoes, flax and cereal grains (Hayes and Laws, 1990; Meister, 1992). It is a grayish powder, practically insoluble in water and in most organic solvents. Mancozeb is

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Table 1. Chemical and physical properties of the Mancozeb (Rohm & Haas Co., 1997)

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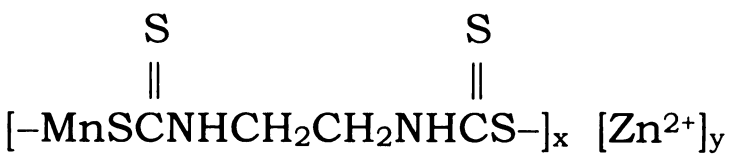

$$[-MnSCNHCH_2CH_2NHCS-]_x \; [Zn^{2+}]_y$$

Common Name: Mancozeb

CAS Register No.: 8018-01-7

Trade Name: Dithane

Molecular Weight: ?

Manufacturer: Rohm & Haas Company

Physical Form: Yellow powdered solid

Odor Characteristic : Musty odor

Melting Point : 192 to 204 °C / 378 to 399°F

Vapor Pressure: Negligible

Specific Gravity (Water = 1) 0.35 to 0.50 g./cc. Bulk Density

Stability Media: Stable; However, keep away from moisture, heat or flame.

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E. Toxicological Properties of EBDCs

The EBDCs, which include mancozeb, are generally considered to have low short-term toxicity to mammals. No toxicological effects were observed in a long term study with rats fed doses of 5 mg/kg (Hayes and Laws, 1990). The major routes of exposure to mancozeb are through the skin or from inhalation (US. EPA, 1987). In spray or dust forms, the EBDCs are moderately irritating to the skin and respiratory mucous membranes. Symptoms of poisoning from this class of chemicals include itching, scratchy throat, sneezing, coughing, inflammation of the nose or throat and bronchitis (Morgan, 1982; OHS, 1991). There is no evidence of 'neurotoxicity', nerve tissue destruction or behavior change, from the EBDCs (Morgan, 1982). However, dithiocarbamates are partially chemically broken down or metabolized to carbon disulfide, a neurotoxin capable of damaging nerve tissue (Hallenbeck and Cunningham-Burns, 1985). The oral LD_{50} for mancozeb ranges from 4,500 to 11,200 mg/kg in rats. When applied to the skin of rabbits, its dermal LD_{50} is 5,000 to 15,000 mg/kg (Berg, 1988; US. EPA, 1987; Hayes and Laws, 1990; Meister, 1992). It is a mild skin irritant and sensitizer and a mild to moderate eye irritant in rabbits (DuPont, 1983). Agricultural workers

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handling crops treated with mancozeb have developed sensitization rashes (Hayes and Laws, 1990). A two–year feeding study on rats indicated that 6.25 mg/kg of maneb in the diet is the no observable effect level (NOEL) for rats. However, the next and highest level that was fed to rats in this two–year study did produce signs of poisoning. A one–year feeding study in dogs concluded that 20 mg/kg/day is a NOEL for dogs. Toxic effects were seen in the dogs at daily doses of 75 mg/kg and 250 mg/kg (DuPont, 1983).

In a three–generation rat study with mancozeb at a dietary level of 50 mg/kg there was reduced fertility but no indication of embryo toxic or teratogenic effects. In another study in which pregnant rats were exposed to mancozeb by inhalation, toxic effects on the embryos were observed only at doses (55 mg/m^3) that were also toxic to mothers (Hayes and Laws, 1990). No teratogenic effects were observed in a three-generation rat study with mancozeb at a dietary level of 50 mg/kg (Hayes and Laws, 1990). Specific developmental abnormalities of the body wall, central nervous system, eye, ear and musculoskeletal system were observed in experimental rats which were given 1,320 mg/kg of mancozeb on the 11^{th} day of pregnancy (NIOSH, 1986). When it was inhaled at concentrations of 0.017 mg/L, mancozeb was not teratogenic to pregnant rats (DuPont, 1983). Teratogenic activity was found in mice given 1,320 mg/kg of maneb (Shepard, 1989).

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Non-tumorigenicity was reported for maneb, zineb and nabam in chronic feeding studies on three strains of mice (Lentza-Rizos, 1990). Mancozeb produced skin tumors in mice at 100 mg/kg body weight, 3 times per week for 31 weeks. Historical examination revealed that these tumors were mostly benign (Shukla *et al.*, 1990). Several studies have shown rapid reduction in the uptake of iodine and swelling of the thyroid (i.e. goiter). Morgan (1982) found that a marked reduction of iodine uptake was measured 24-hours after administration of a large dose of maneb, another EBDC fungicide.

F. Degradation of EBDCs

The EBDCs are generally unstable in the presence of moisture, oxygen, and in biological systems (US EPA, 1992). They are easily degraded in these conditions and several degradation products are formed, including ethylenethiourea (imidazolidine-2-thione, ETU) (Lentza-Rizos, 1990). This rapid degradation lowers the need for concern about the environmental fate of EBDCs and focuses such concern on ETU. ETU has been identified as an impurity in commercial EBDC formulations (Clarke *et al.*, 1951; Bontoyan *et al.*, 1972). Most commercial EBDC formulations contain 0.02-5% of ETU (Bontoyan *et al.*, 1977). It has been reported that ETU occurs as a result of metabolic (Engst and Schnaak, 1974) and chemical (Fishbein and Fawkes, 1965;

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Engst and Schnaak, 1974) alterations of the commercial fungicides. ETU has been identified on a number of different crops which had been field-sprayed with a commercial formulation of EBDC (Yip *et al.*, 1971; Newsome, 1972). Cooking of foods containing EBDC residues also results in the formulation of ETU (Newsome and Laver, 1973; Watts *et al.*, 1974).

Engst and Schnaak (1974) suggested a possible degradation scheme for metabolic derivatives of the ethylenebisdithiocarbamate (Figure 4), speculating that ethylenebisdithiocarbamic acid readily forms ETU under highly alkaline conditions (pH 10.5) and that ETU obtained under these conditions may be formed from ethylenethiuram monosulphide (ETM) by the loss of a molecule of carbon disulfide.

ETU has been known to be a possible degradation product of EBDC fungicides for over 40 years (Clarke *et al.*, 1951; Fishbein and Fawkes, 1965; Bontoyan *et al.*, 1972). It may be formed during manufacture or storage of the EBDCs (Fishbein and Fawkes, 1965) on plants following application of EBDC formulations, or in food containing EBDC residues during cooking and processing procedures (Watts *et al.*, 1974). Pesticide degradation during storage results mainly from hydrolysis and oxidation (Egli, 1982). Photolysis may not be an important degradative reaction during storage since samples are usually stored in the dark at -20°C. Oxidation, especially, is an important reaction for readily oxidizable thio compounds. ETU is degraded from

Figure 4. De

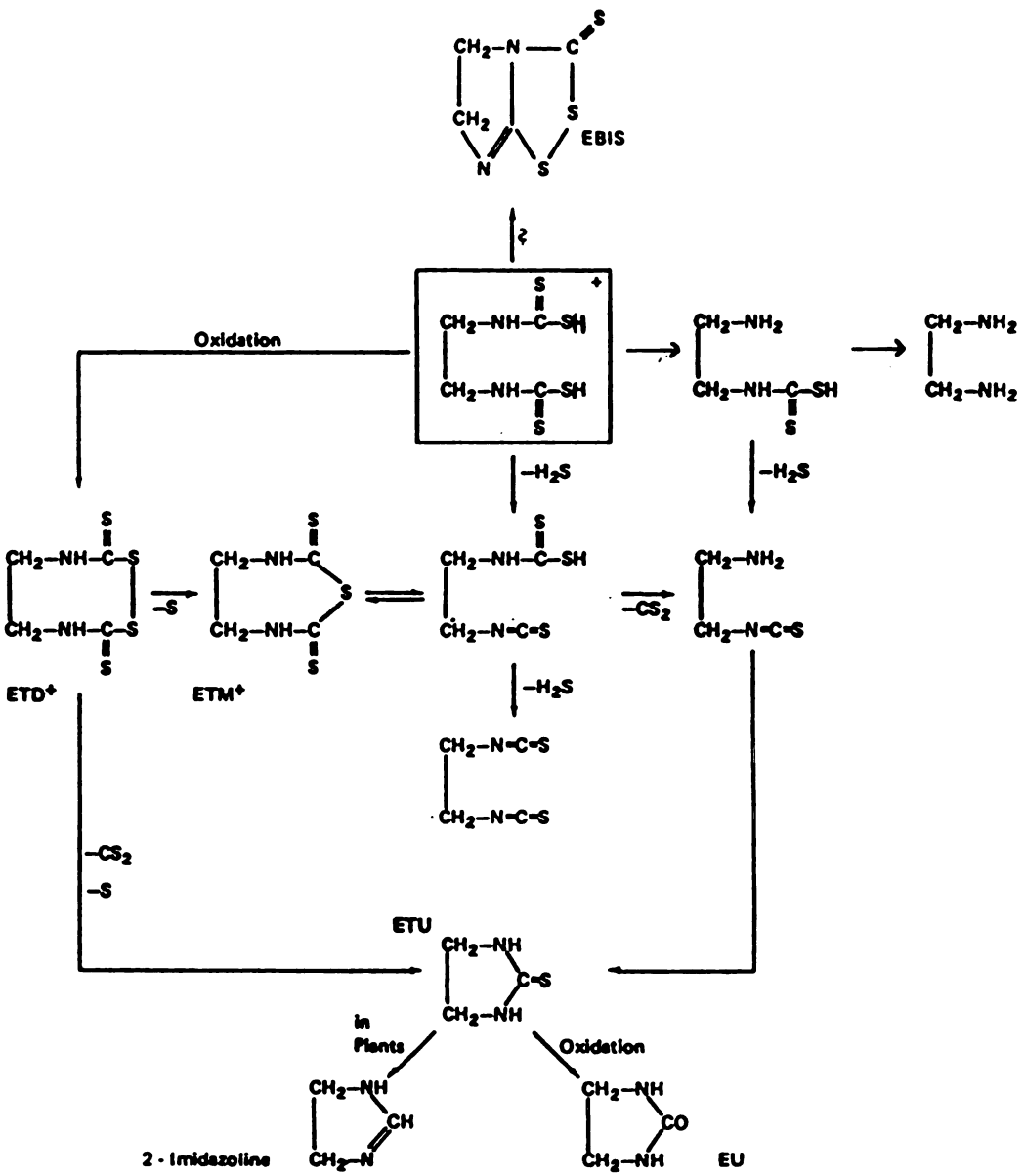

Figure 4. Degradation pathway of EBDCs; + = polymeric products.

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Table 2. Chemical and physical properties of the ETU (Windholz *et al.*, 1983; U.S. EPA 1986)

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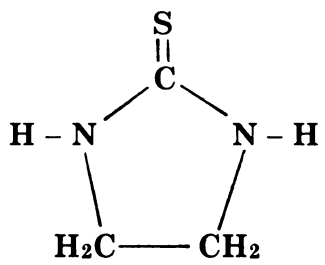

Common Name: Ethylenethiourea

CAS Register No.: 96–45–7

Chemical Name: imidazolidine–2–thione

Molecular Weight: 102.2

Manufacturer: Aldrich Company

Physical Form: White Crystals

Odor Characteristic : Musty odor

Melting Point : 203 °C /400°F

Vapor Pressure: –

Specific Gravity (Water = 1) 0.35 to 0.50 g./cc. Bulk Density

Stability Media: Stable

Solubility in Water (30°C): 20g/L

Percent Volatility : 1% Water

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EBDC fungicides in crops, rice (Rhodes, 1977; Ripley and Cox, 1978; Nash, 1976), aqueous media (Marshall, 1977) and by heat (Newsome, 1976). During storage, ETU has been found to be unstable in certain crops (Uno *et al.*, 1978) and tomato sauce and paste (Ankumah and Marshall, 1984). ETU is soluble in water and readily absorbed and metabolized by plants (Engst and Schnaak, 1974; Newsome and Laver, 1973). It is a common contaminant in technical grade fungicides such as mancozeb, maneb, zineb, and nabam. It may also be formed from EBDC at elevated temperatures, high humidity, environmental degradation or during cooking of food containing EBDC residues (Meneguz *et al.*, 1987). The rate of degradation of EBDC's to ETU is influenced by temperature, available oxygen and pH of the system. (Marshall, 1977).

Several workers have reported the instability of ETU. Cruickshank and Jarrow (1975) reported that ultraviolet light can degrade ETU on a solid substrate such as silica gel to produce 2–imidazolidone as the major product. ETU degradation was especially rapid in the presence of photosensitizers such as acetonaphthone, naphthaldehyde, methylene blue, benzophenone, and crystal violet. Ross and Crosby (1973) found that dissolved oxygen and sensitizers such as acetone or riboflavin degrade ETU in the presence of light. Marshall (1979) reported the oxidative degradation of ETU by hydrogen peroxide and hypochlorite.

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G. Toxicological Properties of ETU

A major toxicological concern surrounding the EBDCs comes from ETU, an industrial contaminant and a breakdown product of EBDCs. No suitable information was found in the available literature on the health effects of ETU in humans. In animal studies, the acute oral LD_{50} for ETU was 1,832 mg/kg in rats (U.S. EPA, 1982). ETU has caused cancer in experimental animals and has been classified as a Group B2 probable human carcinogen based on sufficient evidence from animal studies by the EPA (US EPA, 1992). Because of the report of their carcinogenic (IARC, 1974), mutagenic (Teramoto *et al.*, 1977), goitrogenic (Graham *et al.*, 1975) and teratogenic (Teramoto *et al.*, 1980) effects in laboratory animals, ETU has become a major human health concern among some consumer groups (Lentza-Rizos, 1990). Chernoff *et al.* (1979) demonstrated the teratogenic effects of ETU in Sprague-Dawley rats, CD-1 mice and golden hamsters. Based on the results of this study, the no observable adverse effect levels (NOAELs) for maternal and developmental toxicity were 40 mg/kg/day in the rat, 200 mg/kg/day in the mouse and 300mg/kg/day in the hamster. A 90-day study of the effects of ETU revealed a NOEL of 5 ppm (0.25 mg/kg/day) (Morgan, 1982; Hayes and Laws, 1990; US EPA 1992). Seiler (1973) described ETU as exhibiting weak but significant mutagenic activity in *Salmonella typhimurium*. A 2.5-fold increase in mutation frequencies was seen at

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intermediate concentrations (100 or 1,000 ppm/plate), but at higher concentrations (10,000 and 25,000 ppm), ETU was somewhat lethal to the test colonies resulting in lower relative mutagenic indices. Graham *et al.* (1975) reported that ETU was a follicular thyroid carcinogen in male and female Charles River rats that were fed the compound for 2 years at dietary levels of 250 and 500 ppm (approximately 12.5 and 25 mg/kg/day).

The thyroid appears to be the primary target organ for ETU toxicity in long-term exposure studies. Ulland *et al.* (1972) reported a dose related increased incidence of hyperplastic goiter in male and female rats fed ETU at 175 and 350 ppm (approximately 8.75 and 17.5 mg/kg/day) in their diet for 18 months. An increased incidence of simple goiter was also reported in all treatment groups. Arnold *et al.* (1983) showed that the thyroid effects of ETU administered in the diet for 7 weeks to male and female Sprague–Dawley rats were reversible when ETU was removed from the diet.

H. Formation of ETU During Heat Treatment

The nonbiological degradation of EBDCs to ETU is accelerated by heat treatment and EBDC residues are known to be converted to ETU during normal industrial processing of field-treated produce (Newsome and Laver, 1973; Watts *et al.*, 1974; Marshall, 1977; Phillips *et al.*, 1977).

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The conversion of these surface residues to ETU during cooking, blanching or other processing has been demonstrated on snap beans, tomatoes (Newsome *et al.*, 1975), carrots, spinach (Phillips *et al.*, 1977) and grapes (Ripley *et al.*, 1978).

Ripley and Cox (1978) processed field-treated tomatoes, using simulated commercial methods, into whole pack tomatoes and tomato juice and analyzed these products for EBDC and ETU residues. In the processed products, the EBDC concentration was reduced by 50–75% and the ETU concentration was about the same or slightly elevated compared to the unprocessed fruit levels. They found a good correlation between higher EBDC concentrations and higher ETU concentrations in the same sample. However, the variability of their results indicated a wide rage of conversion due to processing. It should be noted that some samples showed no detectable EBDC residue, but had ETU levels as high as 0.08 mg/kg.

The fate of ETU in the sterile environment of a processed food is controversial. It has been reported that ETU, during a 4-week storage (at 1.0 or 0.1 ppm), decreased to 1% of the initial amount in pickles, 1–5% in apple sauce, 0.1–0.2% in tomato sauce, and 9-12% in spinach (Han, 1977). In contrast, Uno *et al.* (1978) have reported that ETU in tomato puree was stable for up to 200 days. Efficient decontamination procedures are available for the removal of EBDC surface residues from

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tomatoes and green beans prior to processing (Marshall and Jarvis, 1979; Marshall, 1982). A four-minute preprocessing wash with dilute alkaline hydrochlorite followed by a 30-second dip in dilute sodium sulfite was demonstrated to reduce field residues of EBDC and ETU to the limits of analytical significance.

Ross *et al.* (1978) found apples field-treated nine times with mancozeb and metiram contained, respectively, 0.17 and 0.50 mg/kg EBDC residue and 0.01 and 0.03 mg/kg ETU 42 days after the last treatment. Apple juice made from this produce did not contain EBDC residues, but 0.05 mg/kg ETU was present in samples from both pesticide treatments. Dried pomace, which is used as a feed for livestock, was prepared in a laboratory scale experiment by drying the apples at 149°C for 15 hours (a more severe treatment than in commercial pomace production). This dried pomace contained surprisingly high levels of both mancozeb (14.9 mg/kg) and metiram (3.3 mg/kg) residues considering the heat treatment, and high levels of ETU (0.17 and 0.15 mg/kg, respectively). These levels were attributed to the apple peel concentration in the pomace. Apple sauce prepared from apples with the peel and cores removed before grinding and cooking contained residues of EBDC and ETU at the 0.09 and 0.05 mg/kg level, respectively, in the case of mancozeb and 0.09 and 0.04 mg/kg in the case of metiram.

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I. Degradation

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Von Stryk and Jarvis (1978) analyzed tomatoes sprayed with maneb and mancozeb and found EBDC levels between 0.03 and 0.80 mg/kg. ETU was detected only in one sample at 0.03 mg/kg. The tomatoes were processed into juice and canned whole fruits, after washing. The juice contained more fungicide and ETU residues than the canned whole fruits. This was attributed to the fact that in preparation of the juice the skins were not removed, whereas for whole tomatoes they were.

Cabras *et al.* (1987) reviewed the fate of EBDC and ETU residues from vine to wine. According to the data given, most EBDC residues are absorbed by scums and ETU residues may remain in amounts <0.01 mg/kg. However, Kakalikova *et al.* (1988) showed that the amount of ETU varies in relation to the amount of EBDC residues present on harvested grapes. Must and wine produced from grapes treated with mancozeb 14 or 28 days before harvest contained detectable ETU residues, whereas those made from grapes harvested 42 days after treatment did not.

I. Degradation of Pesticide in the Environment

The principal degradation pathways for pesticides in environment can be classified as physical, chemical, and biological factors (Coats, 1991). Under field conditions, a combination of these

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factors usually influences the breakdown of a pesticides and their relative importance depends on the chemical, physical properties of pesticides and their chemical structures. Environmental factors such as moisture, temperature and various management practices also play an important role in degradation of pesticides (Coats, 1991).

The two primary physical agents involved in the degradation process are light and heat. Photolysis of pesticide residues is extremely significant on vegetation, on the soil surface, in water and atmosphere (Zepp, 1991). Direct photo reactions account for only a part of sunlight-induced reactions. Other photochemical reactions which produce reactive transients such as hydroxyl, hydroperoxyl/superoxide, organoperoxyl and other radicals as well as singlet molecular oxygen may influence the fate of pesticides in the environment. Thermal decomposition of the chemicals often occurs. Cold, especially freezing temperatures, can also contribute occasionally to pesticide degradation (Coats, 1991).

Chemical degradation occurs as a result of the various reactive agents in the formulations, tank mixes and in the environment. Water is responsible for considerable breakdown of pesticides in solution, especially in conjunction with extremes of pH. Even slight variance from a neutral pH can cause rapid decomposition of pH-sensitive compounds. Molecular oxygen and its several more reactive forms (e.g., ozone,

superoxide, peroxides) are capable of reacting with many chemicals to generate oxidation products. Chemical oxidations as well as reductions can progress in the presence of inorganic, mostly metallic reagents (Zepp, 1991).

Microorganisms such as bacteria and fungi represent the most important group of pesticide degraders in soil and water (Racke and Coats, 1990). Pesticides can be utilized as a nutrient or energy source by microorganisms, mainly bacteria that have adapted (following repeated exposure) to utilize the pesticide molecule as a source of carbon or nitrogen. This requires an initial hydrolysis of the pesticide, followed by the utilization of at least one metabolite as a nutrient (Figure 5). Plants, invertebrates and vertebrates are further degradation agents. The latter group possesses the most sophisticated enzymatic system capable of biodegrading xenobiotics (Moffat and Whittle, 1999). These systems are most effective in birds and mammals; the spectrum of transformation reactions is very broad and the rates of detoxification and elimination are typically high (Moffat and Whittle, 1999).

Figure 5. Microbial degradation of some pesticides.

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J. Degradation of Pesticides in Solution

(I) Hydrolysis

For many pesticide molecules, hydrolysis is a primary route of degradation. Laboratory studies on the effect of pH and temperature on the breakdown of pesticides in aqueous solution have been conduced to provide information on their relative persistence. Many types of esters are hydrolytically cleaved, yielding two fragments with little or no pesticidal activity. Hydrolysis of esters can occur by hydrolytic decomposition of some esters, while acid–activated hydrolysis typically is induced only by strongly acidic solutions (e.g., pH 3–4).

(II) Chemical Oxidation

Chlorine, chlorine dioxide, potassium permanganate and ozone have been employed historically for the oxidation of organic compounds at water treatment plants and were consequently investigated for their capacity to degrade organic pesticides (Gomma and Faust, 1974; Cash *et al*, 1997).

The capability of one substance to oxidize another is measured by its oxidation potential, normally expressed in volts of electrical energy. The oxidation potential is a measure of the relative ease by an atom, ion, molecule or compound to lose electrons, thereby being converted to a

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Handbook

higher state of oxidation. In general, the higher the oxidation potential, the stronger it is as an oxidant. As indicated in Table 3, HOCl is a stronger oxidizing agent (1.49V) than is free chlorine (1.36V), so that HOCl is actually more desirable when using chlorine as an oxidant in aqueous solution.

Table 3. Oxidation–reduction potentials of various compounds

Reactions	Potential In Volts (E°) 25°C
$F_2 + 2e \rightarrow 2F^-$	2.88
$O_3 + 2H^+ + 2e \rightarrow O_2 + H_2O$	2.07
$H_2O_2 + 2H^+ + 2e \rightarrow 2H_2O$ (acid)	1.76
$MnO_4^- + 4H^+ + 3e \rightarrow MnO_2 + 2H_2O$	1.68
$HClO_2 + 3H^+ + 4e \rightarrow Cl^- + 2H_2O$	1.57
$MnO_4^- + 8H^+ + 5e \rightarrow Mn^{2+} + 4H_2O$	1.49
$HOCl + H^+ + 2e \rightarrow Cl^- + H_2O$	1.49
$Cl_2 + 2e \rightarrow 2Cl^-$	1.36
$HOBr + H^+ + 2e \rightarrow Br^- + H_2O$	1.33
$O_3 + H_2O + 2e \rightarrow O_2 + 2OH^-$	1.24
ClO_2 (gas) $+ e \rightarrow ClO_2^-$	1.15
$Br_2 + 2e \rightarrow 2Br^-$	1.07
$HOI + H^+ + 2e \rightarrow I^- + H_2O$	0.99
ClO_2 (aq) $+ e \rightarrow ClO_2^-$	0.95
$ClO^- + 2H_2O + 2e \rightarrow Cl^- + 2OH^-$	0.90
$H_2O_2 + 2H_3O^+ + 2e \rightarrow 4H_2O$ (basic)	0.87
$ClO_2^- + 2H_2O + 4e \rightarrow Cl^- + 4OH^-$	0.78
$OBr^- + H_2O + 2e \rightarrow Br^- + 4OH^-$	0.70
$I_2 + 2e \rightarrow 2I^-$	0.54
$I_3 + 3e \rightarrow 3I^-$	0.53
$OI^- + H_2O + 2e \rightarrow I^- + 2OH^-$	0.49
$O_2 + 2H_2O + 4e \rightarrow 4OH^-$	0.40

Handbook of Chemistry & Physics, 56th Ed. (1975–76)

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Chlorine Chemistry

Chlorine is presently used as a sanitizer in the food industry for utensils and food-contact surfaces as well as for the treatment of public water supplies. This is used either as gaseous or liquid chlorine or as hypochlorite ion to generate nascent oxygen atoms by the reaction $Cl_2 + H_2O \rightarrow 2HCl + O$. This approach finds broad, international use for disinfection of drinking water and as the final treatment for wastewater. Because of its safety requirements, the use of gaseous or liquid chlorine is usually limited to large facilities with the hypochlorite route being more common at smaller sites. In either case, serious questions have arisen concerning the possible generation of more hazardous chlorinated by-products during the treatment.

Chlorine in water is hydrolyzed very easily to form hypochlorous (HOCl) and hydrochloric acid (HCl). For normal conditions of chlorination, the hydrolysis is essentially completed at pH values >6. In turn, HOCl dissociates with a dissociation constant ranging from 1.6 x 10^{-8} M at O°C to 3.2 x 10^{-8} M at 25°C (Morris, 1966).

$$\mathbf{Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-} \qquad \textbf{Equation 1}$$

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This reaction is essentially complete within a few seconds. In dilute solution and at pH levels above 4, the equilibrium shown in Equation 1 is displaced to the right and very little Cl exists in solution (Laubusch, 1962). Hypochlorous acid is a weak acid (Equation 2) with a dissociation constant at 0°C to 25°C of 1.6 to 3.2 x 10^{-2} M and a pKa of 7.8 to 7.5 (Morris, 1966).

$$\mathbf{HOCl \rightarrow H^{+} + OCl^{-} \quad (pKa = 7.5)} \qquad \textbf{Equation 2}$$

As a result, the chlorine species present in the pH range 3.0–8.0 (the range for most foods) would be HOCl and the hypochlorite ion (OCl^{-}). At pH 5.0, the species distribution would be 99.7% HOCl vs. 0.03% OCl^{-} for a 10^{-2} M chlorine solution at 20°C. At pH 8.0, species distribution shifts to 23.2% HOCl vs. 76.8% OCl^{-} for the same 10^{-2} M solution (Figure 6). At pH 7.5, approximately equimolar concentrations of HOCl and OCl^{-} are present. Generally, HOCl plays a main role in bactericidal and disinfecting function. The bactericidal efficiency of HOCl is nearly 80 times higher than OCl^{-}. The higher the pH, the lower the concentration of HOCl and hence weaker activity and poorer disinfection (Morris, 1966).

Other species besides HOCl includes the hypochlorous hydronium ion, H_2OCl^{+} , the chloronium ion, Cl^{+} and Cl_3^{+} which may be

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present in very low concentrations and/or have very low specific reactivities (Laubusch, 1962).

The tendency for chlorine to acquire electrons is so strong that it may split from the molecule and form the reduced chloride ion by displacement (Wei *et al.*, 1985). This is the basis for the oxidation reactions of HOCl with organic compounds. The antibacterial efficiency and sporicidal effectiveness of chlorine solution has been shown to decrease with increasing pH (Dychdala, 1991). An increase in temperature will decrease the percent of HOCl, and consequently its reactivity with organic compounds (Wei *et al.*, 1985).

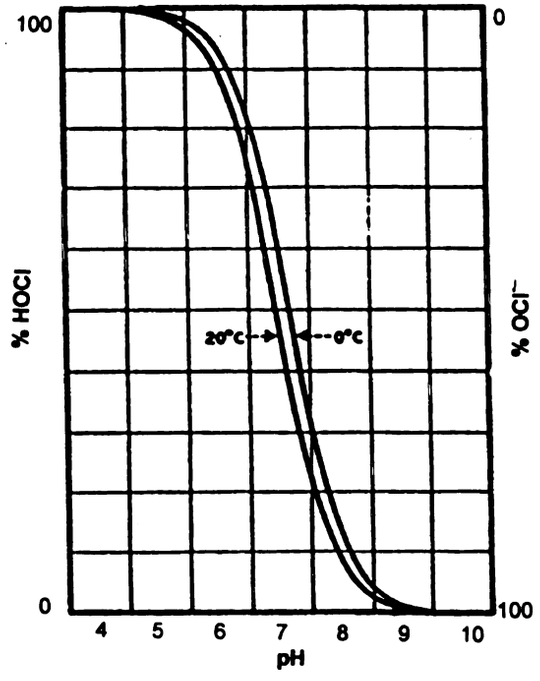

Figure 6. Relative amounts of HOCl and OCl^- formed at various pH levels (Fair *et al.*, 1948).

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Uses of Chlorine

Chlorine as sodium, potassium, or calcium hypochlorite has been used for many years by the food industry as the principal sanitizing and disinfecting agent (Reina *et al,*. 1995). The history of the discovery and use of chlorine in the food industry has been reviewed by Dychdala (1991).

Aqueous chlorine is used extensively in the food industry to sanitize food processing equipment and food containers (100–200 ppm), to rinse raw fruits and vegetables (1–5 ppm), and to cool heat-sterilized canned foods (1–2 ppm) (Foegeding, 1983). Chlorine is also widely used in the fishing industry (Lane, 1974); in washing nutmeats (Smith and Arends, 1976); and in processing seafood (Moody, 1976), poultry (Ranken *et al.*, 1965), and red meats (Kotula *et al.*, 1974). Chlorine gas is used in the flour industry as an oxidizing and bleaching agent to improve the quality of flours (Johnston *et al.*, 1980).

Chlorine, in gaseous form and derivatives such as calcium and sodium hypochlorite, has been used widely in the United States for disinfection of public water supplies and general sanitation. They are powerful disinfectants which are active against a wide spectrum of organisms, and are non–toxic to humans at low concentrations (Dychdala, 1991). Many organic compounds present in water and foods treated with chlorine are subject to chlorination reactions. When chlorine

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is applied to organic molecules, they are changed to molecules with an increased hydrophobicity or lipophilic nature. This in turn often increases the toxicity and bioaccumulation of these compounds (Kopperman *et al.*, 1978). The use of chlorine in food processing is unquestionable in preventing food spoilage and prolonging the shelf life of foods. However, there are potential health hazards connected with the use of chlorine because reaction products are formed that have toxic activity such as mutagenicity, teratogenicity or carcinogenicity. In order to evaluate the possible hazard to human health, more information is needed concerning the level and reactivity of chlorine used in each process, the identification and toxicity of the by-products, and the exposure levels of the population to these compounds.

2) Chlorine Dioxide

Chemistry of Chlorine Dioxide

Chlorine dioxide is a gas that is soluble in water. At low concentrations, the color of the solution is yellow-green, changing to orange-brown at higher concentrations. The odor is similar to chlorine but more pungent. The solubility of chlorine dioxide gas in water is 2.9 g/L at room temperature and 30 mm partial pressure (Latshaw, 1994). Chlorine dioxide is virtually pH independent and is effective at pH 4–10 (Latshaw, 1994). Gaseous chlorine dioxide is sensitive to pressure and

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temperature, so it is impossible to ship in bulk and must be generated on site.

The amount of chlorine in chlorine dioxide is 52.6% by weight (Miller *et al.*, 1978). Since the chlorine atom undergoes five valence charges in the process of oxidation to the chloride ion:

$$ClO_2 + 5e^- = Cl^- + 2O^{-2}$$

the equivalent available chlorine content is 52.6 x 5 = 263%. In effect, this indicates that chlorine dioxide theoretically has about 2.5 times the oxidizing power of chlorine (Miller *et al.*, 1978). Chlorine dioxide achieved faster kill of microorganisms at lower concentrations than did other chlorine-based sanitizers (Aieta *et al.*, 1980). Chlorine dioxide is of equal bactericidal activity to sodium hypochlorite at one-seventh the concentration of hypochlorite, when used for sanitation of poultry processing water (Lillard, 1979). It is shown that oxidation capacity of chlorine dioxide depends upon the acidity and basicity of the solution. The stronger the acidity of solution, the higher the oxidation capacity of chlorine dioxide.

Uses of Chlorine dioxide

Chlorine dioxide offers many advantages over chlorine as a biocide in water systems. Chorine reacts with organic materials to form

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chloroform and trihalomethanes. In contrast, chlorine dioxide does not react with organics, such as ammonia or nitrogenous compounds, so no chloroform or other trihalomethanes are formed. Trihalomethanes are listed as suspected carcinogens and are limited to 10 ppb in drinking water by the U.S., Environmental Protection Agency (Latshaw, 1994).

Chlorine dioxide is used to disinfect public water supplies and is finding application in the food industry. Several reports have addressed the use of chlorine dioxide as a bactericide to reduce bacterial populations both in poultry chiller water and on poultry carcasses (Lillard, 1979; Lillard, 1980). This has proved to be an excellent biocide and an effective oxidant in drinking water, cooling water, waste water, and odor-control applications. This also achieved faster kill of microorganisms at lower concentrations than did other chlorine-based sanitizers (Bohner and Bradley, 1991). Chlorine dioxide has been used as a drinking water treatment agent since 1944. (Aieta and Berg, 1986). In treating drinking water, chlorine dioxide is used for taste and odor control, color removal, iron and manganese oxidation, oxidation of organics, disinfection and for providing a lasting residual in distribution systems. Average dosages of chlorine dioxide can range from 0.1 to 1.5 mg/ L, depending on whether the oxidant is used for final treatment (disinfection) or for pretreatment (removal of algae, Fe, Mn, etc).

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Considerable quantities of chlorine dioxide are used daily for bleaching in the pulp and paper industry. It is also used in large amounts in the textile industry for bleaching and dye stripping, as well as in food processing for bleaching of flour, fats, oils and waxes (Bohner and Bradley, 1991).

3) Ozone

Chemistry of Ozone Technology

Ozone (CAS No. 10028-15-6) is a gas at ambient and refrigerated temperatures. It is a very powerful oxidant that can react with numerous organic chemicals. The oxidation potential of ozone (2.07 V) is higher than HOCl and free chlorine (Table 3). It is a partially soluble in water and, like most gases, increases in solubility as the water temperature decreases (Graham, 1997). It has the unique property of autodecomposition, producing numerous free radical species, the most prominent being the hydroxyl free radical (OH).

The following reaction mechanism was suggested by Alder and Hill (1950).

$$O_3 + H_2O \rightarrow HO_3^+ + OH^- \quad (1)$$

$$HO_3^+ + OH^- \rightarrow 2HO_2^{\cdot} \quad (2)$$

$$O_3 + HO_2^{\cdot} \rightarrow HO^{\cdot} + 2O_2 \quad (3)$$

$$OH^{\cdot} + HO_2^{\cdot} \rightarrow H_2O + O_2 \quad (4)$$

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Ozone is made by rupturing the stable oxygen molecule, forming two oxygen fragments, which can combine with oxygen molecule to form ozone:

$$O_2 \leftrightarrow 2[O]$$

$$2[O] + 2O_2 \leftrightarrow 2O_3$$

The overall equation as follows:

$$2O_3 \rightarrow 3O_2 \qquad (5)$$

Decomposition of ozone can be initiated by hydroxide ions, formate ions, or a variety of other species (Glaze, 1987). A single initiation step can cause the decomposition of hundreds of molecules of ozone before the chain ends. The electrophilic direct ozonolyses by molecular ozone of double or triple bonds and the reactions with OH· radicals are the two most important steps (Stockinger *et al.*, 1994). At high pH condition, the formation of hydroxyl radicals increases and this lowers directly the rate of ozonolyses and vice versa at low pH. As the pH of solutions containing dissolved ozone increases, the rate of decomposition of molecular ozone to produce hydroxyl free radicals also increases, such that at a pH 10 ozone decomposes instantaneously.

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Uses of Ozone

Ozone has been shown to be a more powerful disinfectant than chlorine for deactivation of a very large number of organisms, including the most recalcitrant. Ozonation is approved in the U.S. as generally recognized as safe (GRAS) for treatment of bottled drinking water (FDA, 1995). Ozone has certain characteristics that make it attractive as a sanitizer in food processing, and it is safer than other sanitizer systems. Many applications appear in the food industry. These include the use of gaseous ozone for increasing storage life and dissolved ozone in water for sanitizing surfaces of vegetables, fruits, and other agricultural products. Also, ozone has been used for washing food equipment, food and packaging materials.

The U.S. Fish and Wildlife Service's Coleman Fish Hatchery uses ozonation to inactivate viruses, bacteria, and parasites for protection of spawning salmon (Jennings, 1996). FDA has accepted the use of gaseous ozone up to 0.1 ppm in meat–aging coolers (Ronk, 1975). Ozone decontamination of beef carcasses is also being used in the U.S. (Reagan *et al.*, 1996). Ozone does not remain in water for a very long period of time, thus its use is considered as a process rather than a food additive, with no safety concerns about consumption of residual ozone in food products (Graham, 1997).

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Ozone has been applied in the food industry in Europe for decades, especially in France and Germany, where ozone has been the primary sanitizer for public water system (Graham, 1997). In other European countries, ozone has long been used for various applications, including air purification, storage of meat, fruit, cheese and other products (Easton, 1951). Israel uses ozonation to control postharvest decay of table grapes (Sarig *et al.*, 1996).

4) Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is classified as generally recognized as safe (GRAS) for use in food products as a bleaching agent, oxidizing and reducing agent, and antimicrobial agent (Sapers and Simmons, 1998). Three antimicrobial applications are approved by the Food and Drug Administration (Sapers and Simmons, 1998): treatment of milk for use in cheese, preparation of modified whey, and preparation of thermophile free starch. For these and other food applications, the FDA regulation specifies use levels and requires that residual hydrogen peroxide be removed by appropriate physical and chemical means during processing.

Various experimental antimicrobial applications of hydrogen peroxide for foods have been described, including preservation of fresh vegetables and fruits (Honnay, 1988), control of postharvest decay in table grapes (Forney *et al.*, 1991; Rij and Forney, 1995), washing of fresh

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mushrooms (McConnell, 1991; Sapers *et al.*, 1995) and preservation of salad vegetables, berries, and fresh cut melons (Sapers *et al.*, 1995).

The antimicrobial properties of hydrogen peroxide have long been recognized (Block, 1991). Dilute hydrogen peroxide is used as a topical disinfectant and is available as a consumer product. Hydrogen peroxide vapor shows promise as a sterilizing agent for medical equipment and supplies (Klapes and Vesley, 1990) and for aseptic packaging systems and packaging materials (Wang and Toledo, 1986).

K. Effect of Processing Operations on Pesticide Residues in Foods

Various processing operations on foods give a reduction in the level of pesticide residue (Cash *et al.*, 1997; Siler, 1998). Residues which are loosely held on the surface are removed by washing and blanching, but residues which penetrate the tissues are more difficult to remove from the food. Table 4 presents the effects of processing on the reduction of pesticide residues in fruits and vegetables. The removal of residues from foods depends upon numerous factors, including the type of food, the specific characteristic of pesticides and the severity of the processing operation. Certain residues such as the chlorinated hydrocarbons are located primarily in the lipid materials of animal products and tend to be retained with the lipids during processing (Geisman, 1975).

Table 4.

Food
Apple
Broccoli
Grapes
Orange
Peaches
Pears

Table 4. The effects of processing on pesticide residues in fruits and vegetables (Cash *et al.*, 1997; Fahey *et al.*, 1971; Farrow *et al.*, 1969; Ong *et al.*, 1996; Siler, 1998; Tafuri *et al.*, 1970)

Food	Residue	Process	% Reduction
Apple	Captan, Carzol, and Guthion	Chlorine wash (50 and 500 ppm)	80–100
		Ozone wash (0.25 ppm)	29–42
	Captan and Azinphosmethyl	Chlorine wash (500 ppm)	87–100
		Detergent wash (2% SDS)	50–80
		Peeling, steaming	
	Propargite	Ozone wash (1, 5, 10 ppm)	30–100
		Peeling, steaming	
Broccoli	Carbaryl	Washing (detergent)	77
		Blanching, washing	99
	Parathion	Water wash	None
		Detergnet wash	30–33
		Blanching, washing	10
		Hand washing	None
		Washing, blanching, freezing	10
	Malathion	Washing, cooking	7–34
		Storage (6 months frozen)	45–77
Grapes	Chlorcholine chloride	Wine making	None
Orange	Guthion	Washing	30
Peaches	Gordona	Lye peeling	99
Pears	Gordona	Canning and peeling	98

Table 4 (C

Food
Potatoes
Spinach
Tomatoes

Table 4 (Cont'd)

Food	Residue	Process	% Reduction
Potatoes	DDT	Peeling (home)	91
		Washing (5% lye) and peeling	94
		Washing (15% lye)	90
		Washing, blanching, canning	96
		Washing, commercial	20
Spinach	DDT	Detergent washing	48
		Blanching, washing	60
		Washing, blanching, canning	91
	Carbaryl	Washing, blanching, canning	99
		Detergent washing	87
	Diazinon	Blanching, washing	60
		Water (detergent) washing	None
	Parathion	Blanching, washing	71
		Water washing	9
		Detergent washing	24
		Hand washing (home)	39
		Washing, blanching, canning	66
Tomatoes	Azodrin	Cold wash	36–77
		Hot lye peel	93
	Carbaryl	Detergent washing	97
		Storage (55°F; 7 days)	30
		Cooking	69
		Home canning	92

Table 4 (C

Food
Tomatoes
Tomato
Juice

Table 4 (Cont'd)

Food	Residue	Process	% Reduction
Tomatoes	Malathion	Water washing	36–79
		Detergent wash	90–95
		Cooking	90
Tomato Juice	Carbaryl	Home canning	67

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Most commodities for processing are subjected to a number of unit operations depending on both the commodity and the finished product. Specific unit operations that may affect pesticide residues include inspecting, washing, blanching, peeling and retorting or pasteurizing. Inspection of the raw product with subsequent removal of damaged material could reduce residue. Washing including rinsing is the one of common unit operation to the preparation of nearly all fruits and vegetables for processing. Various physical and chemical parameters of the operation are important in reducing pesticide residues. The physical aspects included soaking time, soaking temperature, agitation during soaking, rotation of commodities under spray rinse, number and type of nozzles, spray rinse pressure and volume. The chemical aspects of washing are wetting agent type and chemical concentration. Blanching is a mild heat treatment or partial cooking usually employed with vegetables. The blanching operation is usually accomplished either in steam or hot water. This operation may also accomplish some washing of the product. Peeling, when applicable, would remove any surface contaminants. The main disadvantage is that not all commodities can be peeled. Peeling may be done by hand, mechanically or chemically. Most pesticides which appear to be heat unstable may be degraded when heated in the presence of food products. Any unit operation which employs heat offers potential in reducing residues. Home preparation

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and cooking of many products also aids in reducing or removing residues (Geisman, 1975).

L. Chemical By-Products and Degradation Pathways of Pesticides

Knowledge of the fate of pesticides in the environment is critical to environmental risk assessments and management decisions. The public demands a safe environment relatively free from toxic chemicals and pesticides used in agricultural industries. In particular, there is a need for understanding the fate and pathways of chemicals in the environment to assess the exposure to humans and animals.

The fate of pesticide through processing of raw agricultural commodities to finished foods is poorly understood and very few published studies address this issue. Chlorination of drinking water is known to produce some chemicals that cause cancer in laboratory animals. These are chloroform, bromodidichloromethane, and MX [3–chloro–4–(dichloromethyl)–5–hydroxy–2(5H)–furanone] (Richardson, 1998). Because of these concerns, alternative disinfectants are being explored as disinfectants for food processing and drinking water.

Use of ozone, chlorine dioxide and chloramine as alternatives to chlorine for treatment of drinking water is increasing, mainly because they produce fewer chlorinated disinfection by–products (DBPs). Because the alternative disinfectants do not form appreciable levels of these

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DBPs, they are gaining in popularity and use. However, it is unknown whether they produce compounds as harmful or more harmful than those produced by chlorine. No research is currently being conducted to identify DBPs formed when these alternative disinfectants are used to treat foods. Because of the similarity in precursor material, it is possible that many of the by–products formed in the processing of fruits and vegetables will be similar to those formed in drinking water treatment.

CHAPT

CHAPTER I. STUDIES ON THE DEGRADATION OF PESTICIDES IN A MODEL SYSTEM

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INTRODUCTION

No one can doubt the efficacy of pesticides for the protection of crops in the field, thereby providing us with abundant, inexpensive, wholesome and attractive fruits and vegetables. However, the widespread use and misuse of the toxic pesticides created an awareness of the potential health hazards and of the need to protect the consumer from residues in foods. Today, the total health risks presented by pesticide residues in our food supply remain unknown. Experimental data indicate that of the 300 pesticides used on food, as many as 71 are known, probable or possible human carcinogens (Hajslova, 1999). Other pesticides in food have been shown to cause neurotoxicity or reproductive toxicity. Children may be uniquely vulnerable because their food intake is a larger percentage of their body weight than adults.

EBDC compounds have been employed as fungicides and they are widely used on a large variety of small fruits and vegetables. The nomenclature of these agents comes from the metal cations with which they are associated. The EBDCs registered for food uses in the U.S. are mancozeb, maneb, penncozeb, ferbam and polyram. Although their toxicity is negligible in animal feeding studies even at high doses, EBDCs are subject to decomposition, and yield ethylenethiourea (ETU) as one of

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their degradation products. ETU is toxicologically significant because of goiterogenic, oncogenic and teratogenic effects after being applied to laboratory animals (Lentza–Rizos, 1990). ETU is present in nearly all commercial formulations of EBDCs (Bontoyan *et al.*, 1977). Some evidence might point to hazards from other breakdown products of EBDCs, such as carbon disulfide, as a neurotoxicant. It is also known that dithiocarbamates can bind various divalent metal to form more lipophilic complexes capable of entering the central nerve system (Ecobichon, 1994).

The present study was focused on the use of chlorine, chlorine dioxide, ozone and hydrogen peroxyacetic acid in the degradation of pesticides in a model system solution. Calcium hypochlorite and chlorine dioxide, common disinfecting and bleaching chemicals used in the food industry, are potent oxidizing and chlorinating agents. Ozone has been shown to be a more powerful disinfectant than the most commonly used chlorine for deactivation of a very large number of microorganisms and pesticide residues (Ong *et al.*, 1996). Hydrogen peroxide has been shown to have bleaching, oxidizing and antimicrobial properties (Sapers and Simmnos, 1998). Hydrogen peroxide is unstable in solution but combined with acetic acid, it forms peroxyacetic acid or hydrogen peroxyacetic acid (HPAA), which is a fairly stable compound.

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The objective of this study was to determine the effectiveness of different chemical oxidants on the degradation of mancozeb and ETU in an aqueous solution model system, using calcium hypochlorite, chlorine dioxide, ozone and HPAA treatment. The optimum parameters determined in the laboratory studies were then applied to apples and apple products.

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MATERIALS AND METHODS

MATERIALS

A. Reagents

(I) Solvents

All organic solvents used for preparation of stock solutions, in sample extraction and high performance liquid chromatography (HPLC) were distilled-in-glass grade. Acetone and methylene chloride were obtained from J. T. Baker, Co. (Phillipsburg, NJ).

(II) Chemicals

Mancozeb standard was obtained from Rohm & Haas (Philadelphia, PA). ETU standard was obtained from Aldrich Co. (Milwaukee, WI). The stock solutions of mancozeb and ETU were prepared in distilled water at concentration of 100 μg/100 ml. The standards were protected from light and stored in refrigerator at 4°C. Chlorine solutions were prepared from calcium hypochlorite (Milwaukee, WI). Sodium thiosulfate, sodium sulfate, potassium iodide, potassium indigo trisulfonate were all reagent grade.

B. Glassware

All glassware was thoroughly washed with detergent and warm water, then rinsed with distilled water. The glassware was then rinsed with acetone and placed in an oven at 400°C overnight before use.

METHODS

Solution studies were conducted in a model system to determine the effect of (i) calcium hypochlorite at three concentrations (50, 250 & 500 ppm), chlorine dioxide at two concentrations (5 & 10 ppm), ozone at two concentrations (1 & 3 ppm), and hydrogen peroxyacetic acid at two concentrations (5 & 50 ppm) (ii) three pH's: 4.6, 7.0, and 10.7 (pH 4.6, 0.2 M citrate–phosphate; pH 7.0, 0.2 M sodium–phosphate; and pH 10.7, 0.2 M carbonate–bicarbonate) (iii) two temperatures: low (10°C) and ambient temperature (21°C). Degradation of the mancozeb was studied over a 30–minute period because the typical water contact time in a commercial plant is about 10–15 minutes and under normal conditions would rarely exceed 30 minutes. There were three replications per treatment. Samples were taken at appropriate intervals for analysis of mancozeb and ETU residues.

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A. Sample Preparation

(I) Calcium Hypochlorite

For the chlorine source, calcium hypochlorite stock solution (5000 ppm) was added to each pH solution to bring the final chlorine concentration to 50, 250 or 500 ppm. Each pH solution was spiked with the mancozeb stock solution to give a final concentration of 2 ppm. Total available chlorine was determined by total residual chlorine and measured using the iodometric method (Standard Methods for Examination of Water and Wastewater, 1987). 10 ml, 20 ml and 100 ml samples from the 500, 250 and 50 ppm chlorine sample solution, respectively, were pipetted to Erlenmeyer flasks containing 5ml acetic acid and 1 g potassium iodide. The stirred samples were titrated with 0.01N sodium thiosulfate, $Na_2S_2O_3$, until the endpoints were achieved.

The total residual Cl_2 was determined using the formula :

$$\textbf{mg } Cl_2 \textbf{ / L = [(A } \pm \textbf{ B) x N x 35450] / ml of sample}$$

where A is the amount $Na_2S_2O_3$ titrated for the sample (in ml), B is the amount $Na_2S_2O_3$ titrated for the blank (in ml) and N is the normality of $Na_2S_2O_3$ (0.01 N).

(II) Chlorine Dioxide

Chlorine dioxide (ClO_2) was generated in the laboratory using the manufacturer's (S.C. Johnson Professional, WI.) instructions as follows: 100 mls of the stock 2% Oxine FP solution were added to a 200 ml French square screw capped bottle. Twenty five mls of 75% w/w food grade phosphoric acid were added, sealed and allowed to generate chlorine dioxide for 5 minutes with a magnetic stirrer to ensure though mixing. After 5 minutes, the concentrated chlorine dioxide was transferred into 5 gallons of each pH solution in a closed container to serve as a stock solution. For 5 or 10 ppm of chlorine dioxide, 2 or 4 liters of stock solution, respectively, were diluted to 10 gallons with each pH buffer solution.

The final concentration of chlorine dioxide was determined using the HACH chlorine colorimeter (Model CN–66, Cat. No. 2231–01, HACH Co., Loveland, CO.) before and after each sampling run. A 1:2000 dilution of unactivated Oxine FP solution was used as a control blank. Ten mls of the control stock solution or test solutions were transferred into test solution vials. Two or three drops of Hach Glycine reagent and one "free chlorine DPD" were added into the vials and then mixed gently. After 1 minute, the blank solution was read using the colorimeter and

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(III) Ozone

Ozone was bubbled through a glass sparger (i.e. bubbles of approximately 10 mm i.d.) into 990 ml of distilled water at the appropriate temperature adjusted by a circulating water bath and pH adjusted by the addition of standard buffer solutions under 25 psi at 15 SCFH of oxygen until the desired ozone concentration (1 or 3 ppm) was attained. One hundred ml of ozonated water was spiked with mancozeb to give a final concentration of 2 ppm.

Ozone detection and monitoring were performed using the indigo colorimetric method as described in Standard Methods for the Examination of Water and Wastewater (1987). All reagents were prepared just prior to use. The ozone concentration was monitored before and after each sampling run. The ozonated water was collected into a 100 ml volumetric flask containing 10 ml of the indigo reagent to minimize loss of ozone. A separate volumetric flask was filled with distilled water containing 10 ml indigo reagent to serve as a blank. The solutions were mixed thoroughly and the absorbance of each solution was immediately measured at 600 nm in a 1 cm cell.

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The concentration of ozone, in mg/L, was calculated using the formula:

$$\text{mg } O_3/L = (1000 \times A) / (f \times b \times V)$$

where A is the difference in absorbance between sample and blank solution, b is the path length (1 cm), V is the volume of the sample (90 ml), and f is a constant of 0.42.

(IV) Hydrogen Peroxyacetic Acid Study

An appropriate amount of peroxyacetic acid stock solution was added to each pH solution to bring the final peroxyacetic acid concentration to 5 or 50 ppm. Each pH solution was spiked with mancozeb stock solution to give a final concentration of 2 ppm.

Total residual peroxyacetic acid was measured using the POAA test kit (Ecolab Inc., 1997). The procedure is as follows;

Each solution was less than 90°F prior to testing. Vials were rinsed with solution to be tested then filled with 10 ml of the test solution. Five drops of potassium iodide were added and mixed. Five drops of phosphoric acid were added, mixed and then five drops of starch indicator were added with vigorous mixing. Sodium thiosulfate (N/200) was added, one drop at a time, counting drops and mixing between drops, until blue color just disappeared.

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Calculation: Each drop of thiosulfate N/200 equals 1ppm residual peroxyacetic acid (POAA).

Total residual POAA

= (Drops for solution-drops for blank) x 1 ppm residual POAA

B. Pesticide Residue Analyses

(I) Mancozeb

Mancozeb residues were analyzed as carbon disulfide (CS_2) by gas liquid chromatographic headspace analysis (Ahmad *et al*, 1995). Twenty mls of sample were transferred at 0, 5, 15, and 30 minutes interval into sample bottles. A 0.5% 0.1 M sodium thiosulfate solution was added to the samples at the appropriate time to quench the reaction. Forty mls of 1.5% stannous chloride in 5 M HCl were added and immediately sealed with a crimped septum. Fifty µls of a 1 mg/ml thiophene solution were injected into each bottle and incubated at 70–80°C in a water bath for 15 minutes. Bottles were removed and agitated for 2 minutes by hand. Bottles were replaced in the water bath with repeated shaking for 1 hour. A 100 µl sample was removed with a gas tight syringe from the bottle headspace, and injected into the GC.

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(II) ETU

ETU residues were determined using a modification of the HPLC method published by Ahmad *et al.* (1995). Twenty mls of sample were weighed into an Erlenmeyer flask, then 8 g of potassium fluoride and 0.6 g of ammonium chloride were added. This mixture was extracted with 50 ml methylene chloride 2 times. The methylene chloride layer was passed through a bed of 25 g anhydrous sodium sulfate (120°C for at least 12 hr), collected in a Zymark Turbovap tube and evaporated to dryness on an automated Zymark Turbovap evaporator (Zymark Inc., Hopkin, MA) at 40°C. The residue was dissolved in 3 ml distilled water and 50 µls were injected into an HPLC column.

C. Chromatographic Analyses

(I) Mancozeb

Mancozeb residues were detected and quantified using a Hewlett Packard Series II 5890 gas chromatograph (GC) equipped with a flame photometric detector (FPD) in the sulfur mode. The GC was equipped with a Supel-Q-Plot fused silica capillary column (30 m long x 0.53 mm ID) with a film thickness of 0.25 µm (Supelco Inc., Bellefonte, PA). The oven temperature was 80°C, while the injector and detector temperatures were 230°C and 300°C, respectively. Helium and nitrogen were used as the GC carrier gas and makeup gas, respectively. Carrier

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gas flow through the column was 20 ml/min. Integration was carried out with HP Chemstation software interfaced to the GC.

(II) ETU

ETU residues were detected and quantified using a Waters liquid chromatograph with a Hypersil BDS C_{18} column (250 mm x 4.6 mm, 5 μm particles), a Hypersil BDS C_{18} guard column (10 mm x 4.6 mm, 5 μm particles) and UV detector set at 240 nm. The mobile phase was 0.72% butylamine in distilled water at pH 3.0–3.2. A M–45 Waters HPLC pump (Waters Associates, Inc., Milford, MA.) was used for solvent delivery at a flow rate of 0.5ml/minutes. After the system was stabilized (about 1 hour from initial warm–up), 75 μl samples were injected via Rheodyne syringe loop injector (50 μl loop) for analysis. Integration was carried out using 3390 A Hewlett Packard integrator.

D. Calculation of Pesticide Residue Concentration

Mancozeb and ETU residue concentrations in solution were calculated based on the area of the integrated peaks of the samples compared with known concentrations of analytical standard of the respective pesticides. Standard curves of the mancozeb and ETU were plotted and least square linear regression was obtained using a Microsoft Excel (Microsoft Corporation, Redmond, WA) software. Appendix 1 and 2

show examples of standard curves for mancozeb and ETU standard of known concentrations.

The residue concentrations were calculated based on the following formula:

(a) Mancozeb residue in μg/ml

$$ppm = \frac{\text{ng Mancozeb}}{\text{mg sample injected}}$$

where, ng Mancozeb was derived from standard curve

$$\text{mg sample injected} = \frac{20\ \text{g}}{\text{headspace volume sample – containing reaction vial} \times \mu\text{L injected}}$$

where, headspace volume of sample – containing reaction vial = 40 mL

(b) ETU residue in μg/ml =

$$\frac{\text{Conc. of ETU in sample extract based on std. curve}(\mu g/g) \times \text{Vol. final extract (3 ml)}}{\text{Weight of sample analyzed (20 g)}}$$

E. Statistical Analyses

All determinations were replicated three times. Mean standard deviations, mean square errors, two factor ANOVA, correlation and interaction of main effects were calculated using Sigmastat computer software 1.0 (Jandel Corp., San Rafael, CA). Appropriate comparisons

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were made using Student–Newman–Keuls Method for multiple comparisons. A $p<0.05$ was considered statistically significant.

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RESULTS & DISCUSSION

A. Chromatographic Analyses

(I) Mancozeb

A variety of analytical methods have been developed for mancozeb analysis. As for many other dithiocarbamate pesticides, one of the most widely used procedures for determining EBDC residues is the headspace gas–liquid chromatographic (GLC) method. The matrix was heated with hot acid and degraded the active ingredient to carbon disulfide (CS_2). Released carbon disulfide was detected and measured directly by GLC headspace analysis linked to flame photometric detector (FPD). To improve its sensitivity and resolution, thiophene was incorporated as an internal standard. In the GLC analysis, carbon disulfide appeared as a single sharp peak at a retention time of 5.1 minutes. Figure 7 shows a typical chromatogram of mancozeb standard at a concentration of 1 ppm in distilled water, while Figure 8 shows an example of a chromatogram of a sample in a 3 ppm ozonated water at pH 7.0 solution, room temperature and sampled at 5 minutes. The standard curve shown in Appendix 1 was representative of the standard curves used to calculated mancozeb concentration in the sample solutions. The correlation coefficients (R^2) for linear regression of the standard curves

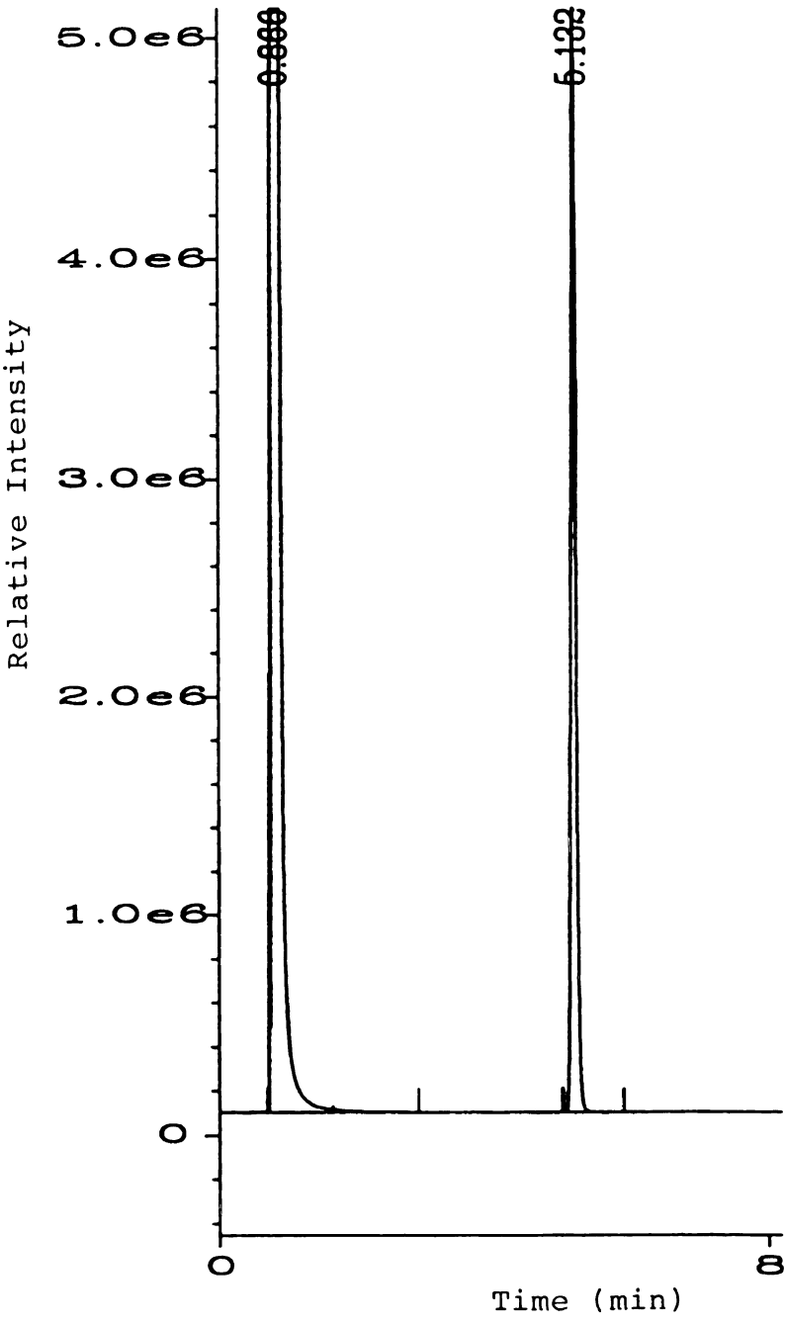

Figure 7. GC chromatogram of a Mancozeb standard

1) 1.0 ppm standard in distilled water

2) Rt = 5.1 minutes

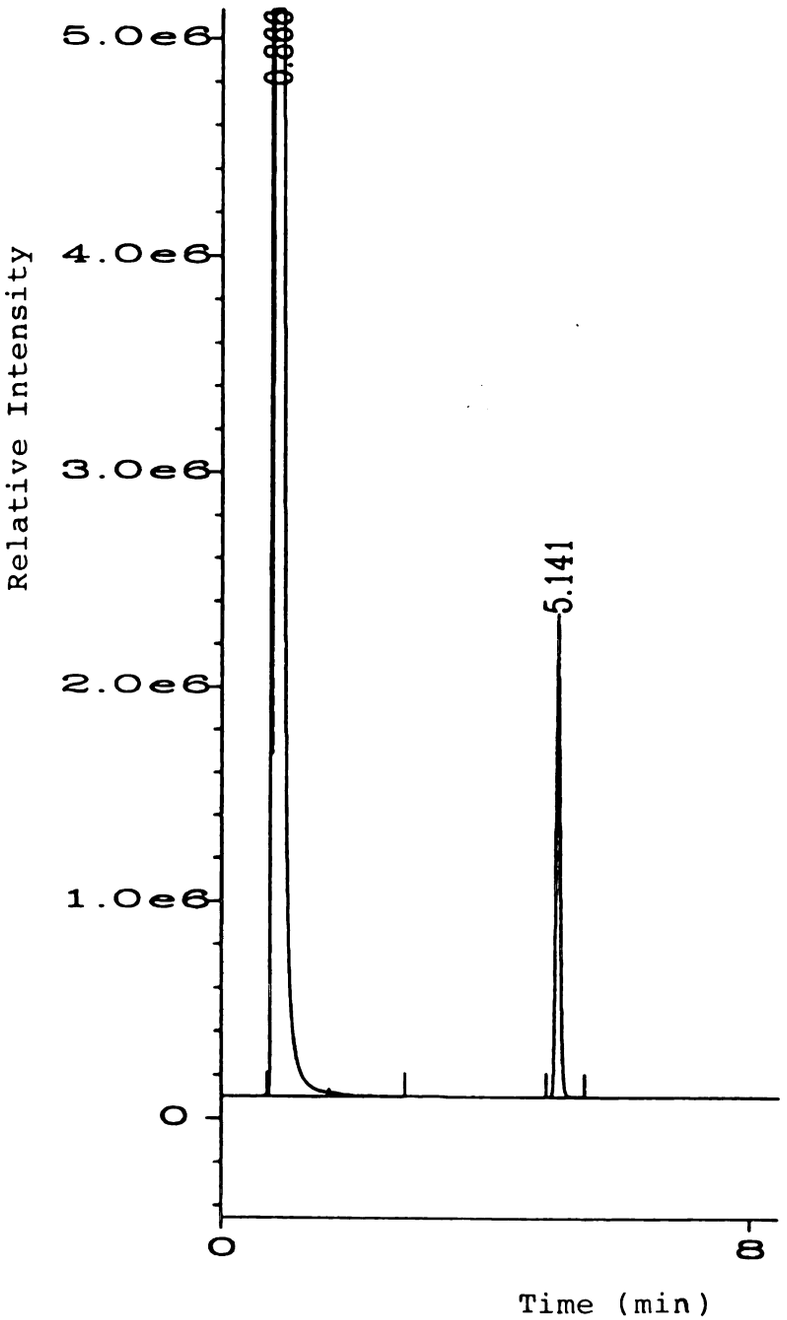

Figure 8. GC chromatogram of a Mancozeb sample

1) 3ppm O_3 in pH 7.0 at ambient temp. ; reaction time = 5 minutes
2) Rt = 5.1 minutes

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were between 0.91 and 0.99, showing that the response was linear over the concentration range of 1 to 500 μg.

(II) ETU

GLC methods have been the widely used for the determination of ETU, because of its high sensitivity and specificity obtained by the use of a number of different detectors. It must be pointed out, however, that many workers have encountered difficulties with direct analysis of ETU at low residue levels and some have demonstrated that the results obtained using GLC must be treated with caution because of the possibility of breakdown of any EBDCs and intermediate breakdown products present under the conditions used for gas chromatography. Comparisons of the results obtained on analysis of formulations using both GLC and HPLC have shown that GLC may give abnormal results (Bottomley *et al.*, 1985). HPLC gives a better estimate of the ETU content because of the lower operating temperatures as compared to the high temperatures involved in GLC which may give rise to the degradation of co-extractives on the column to form ETU. Consistently higher results were obtained using GLC than by HPLC.

ETU was detected using liquid chromatography linked to a ultraviolet (UV) spectrophotometric detector. ETU appeared as a peak with a retention time of 10.4 minutes. Figure 9 shows a typical

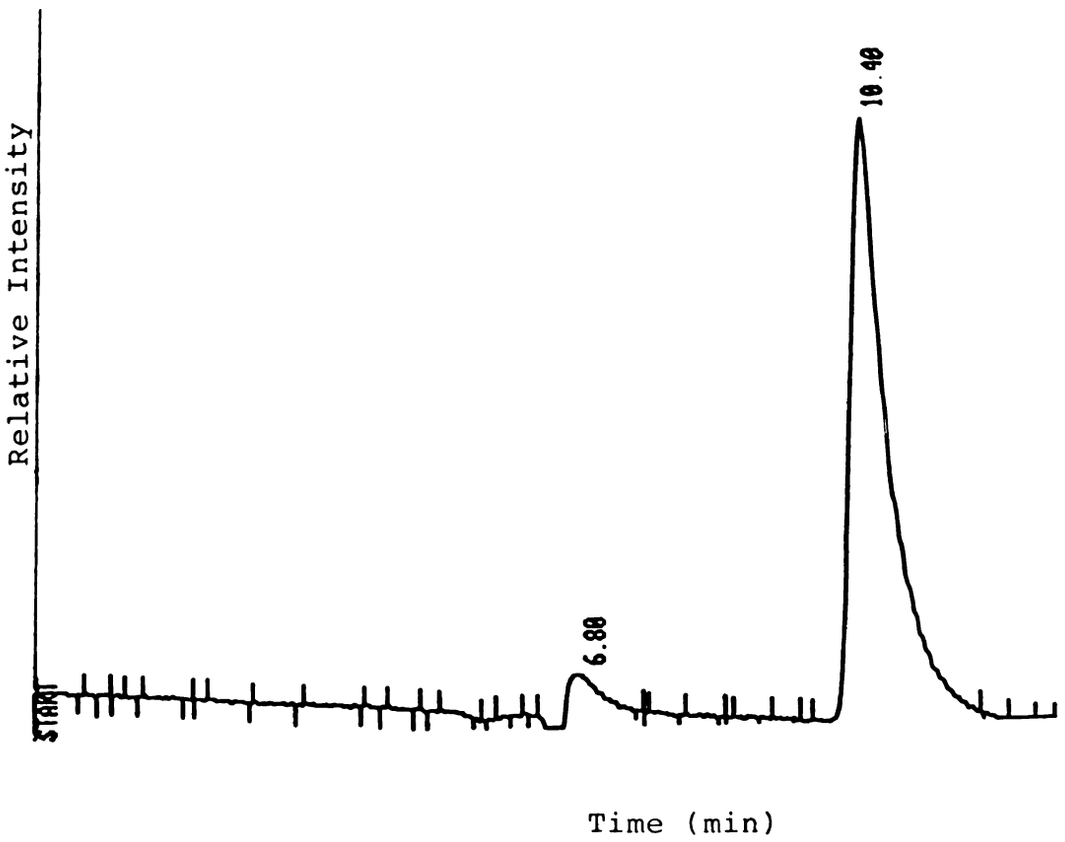

Figure 9. HPLC chromatogram of a ETU standard

1) 1.0 ppm standard in distilled water
2) Rt = 10.4 minutes

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chromatogram of ETU standard at a concentration of 1 ppm in distilled water, while Figure 10 shows an example of a chromatogram of a control sample in pH 7.0 solution, ambient temperature and sampled at 5 minutes. Standard curves for ETU standards were plotted, and a typical curve is shown in Appendix 2. The correlation coefficients (R^2) for the linear regression of the curves were between 0.94 and 1.00.

B. Degradation of Mancozeb in Solution

(I) Degradation of Mancozeb by Hydrolysis

Mancozeb was stable at pH 7.0 at both 10°C and 21°C with very little degradation due to hydrolysis. Between 95–99% (10°C) and 95–97% (21°C) residual mancozeb remained after 30 minutes. Mancozeb was relatively less stable at pH 4.6 and 10.7, with about 78 and 80% remaining, respectively after 30 minutes at ambient temperature (Figure 11). This indicates mancozeb is less stable under basic and acidic conditions than neutral condition. Appendix 3 shows raw data for mancozeb residues in a model system under various oxidizing agents, temperature and pH conditions.

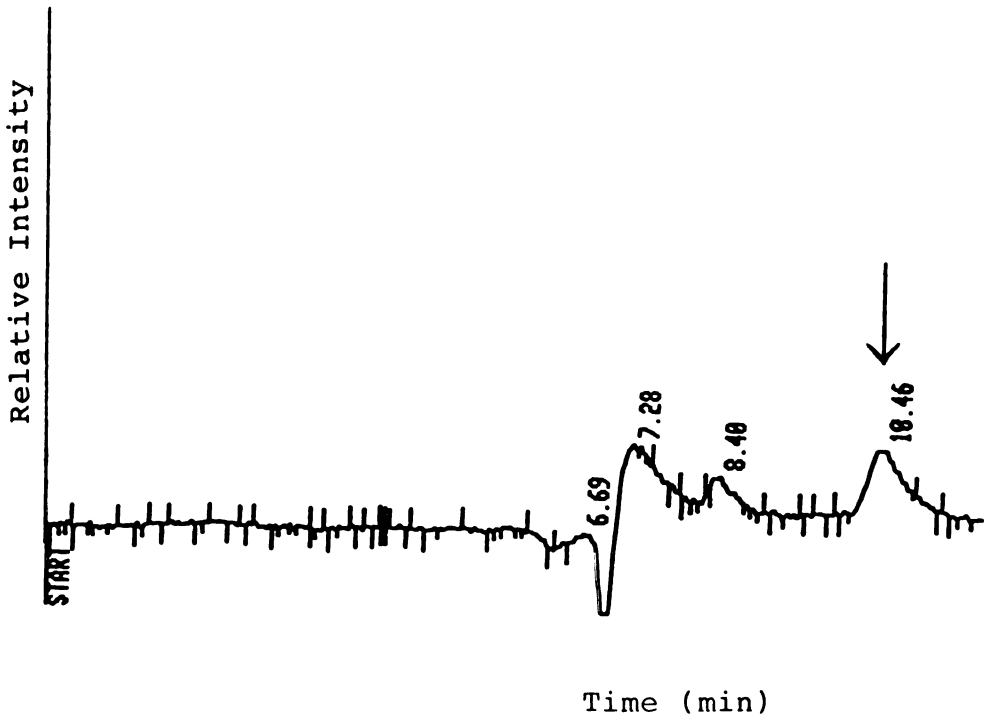


Figure 10. HPLC chromatogram of a ETU sample

1) Control in pH 7.0 at ambient temp. ; reaction time = 5 minutes
2) Rt = 10.46 minutes

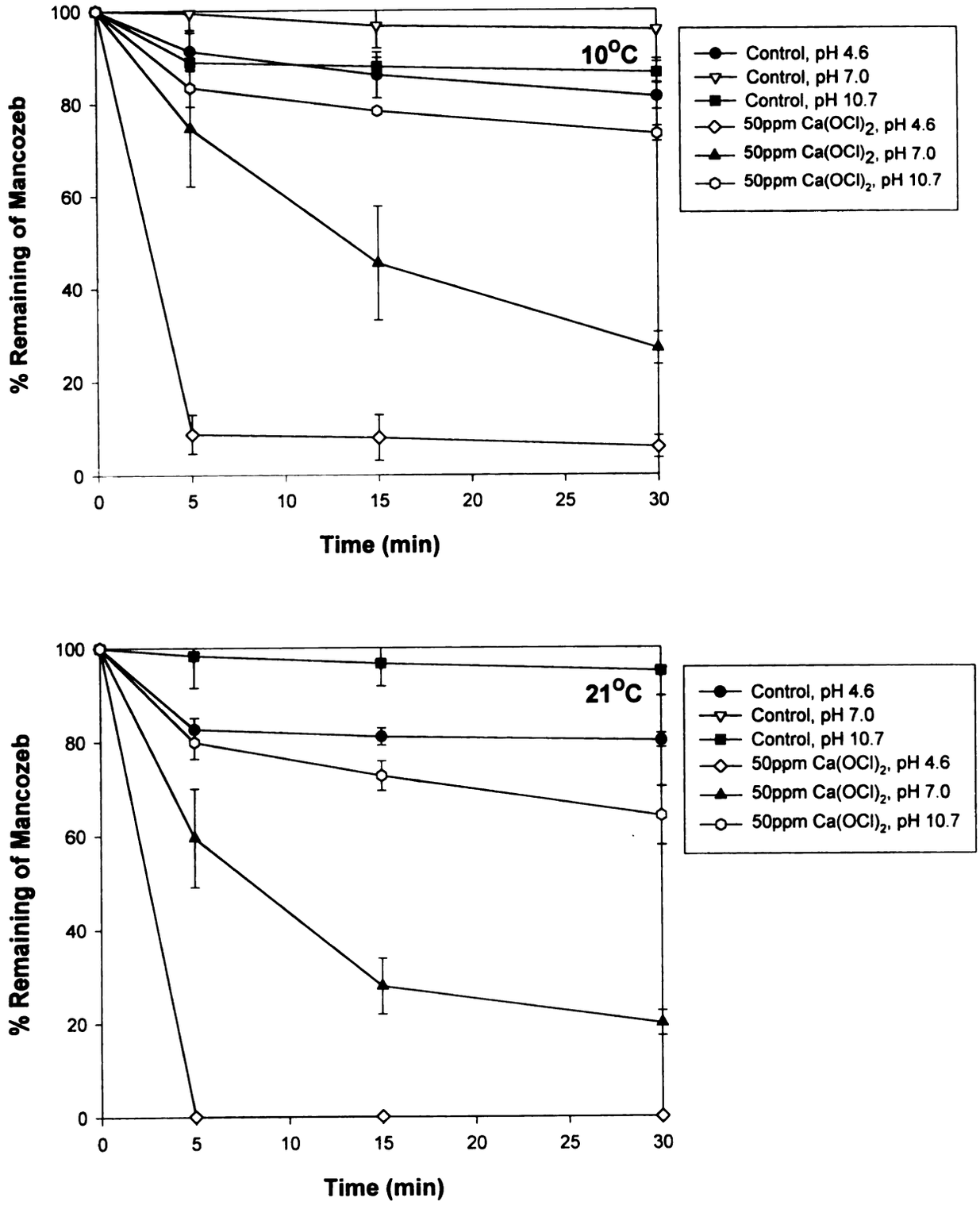


Figure 11. Effect of 50 ppm $Ca(OCl)_2$ on the Degradation of 2 ppm Mancozeb at 10 and 21°C.

(II) Degradation of Mancozeb by Calcium Hypochlorite

Degradation of mancozeb by calcium hypochlorite solution was greatest at pH 4.6 and decreased with increasing pH (Figures 11–17). The chlorine treatment at pH 10.7 was the least effective at both 10°C and 21°C. Its degradation was only about 27 and 40% after 5 minutes at 50 ppm calcium hypochlorite (Figure 11). In 50 ppm calcium hypochlorite solution, mancozeb was completely degraded at pH 4.6 after 5 minutes at ambient temperature (Figure 12). The 50 ppm chlorine treatment at pH 10.7 was the least effective, with degradation only about 20% and 36% after 5 and 30 minutes, respectively. Low temperature decreased the degradation of mancozeb at all pH ranges during the entire sampling period (Figures 11–12). Chlorination at 50, 250 and 500 ppm significantly ($p<0.05$) increased the rate of degradation of mancozeb in all three pH treatments and at both temperatures. No mancozeb remained with 250 and 500 ppm calcium hypochlorite treatments at pH 4.6 and ambient temperature after 5 minutes (Figures 13–16). At pH 10.7, 50% and 30% mancozeb residues remained after 5 minutes at 250 and 500 ppm calcium hypochlorite, respectively at ambient temperature (Figures 13, 15). The effects of pH on the degradation of mancozeb in solution are illustrated in Figure 17. Again, the most effective pH for the degradation of mancozeb with chlorination was pH 4.6, while pH 10.7 was the least effective treatment.

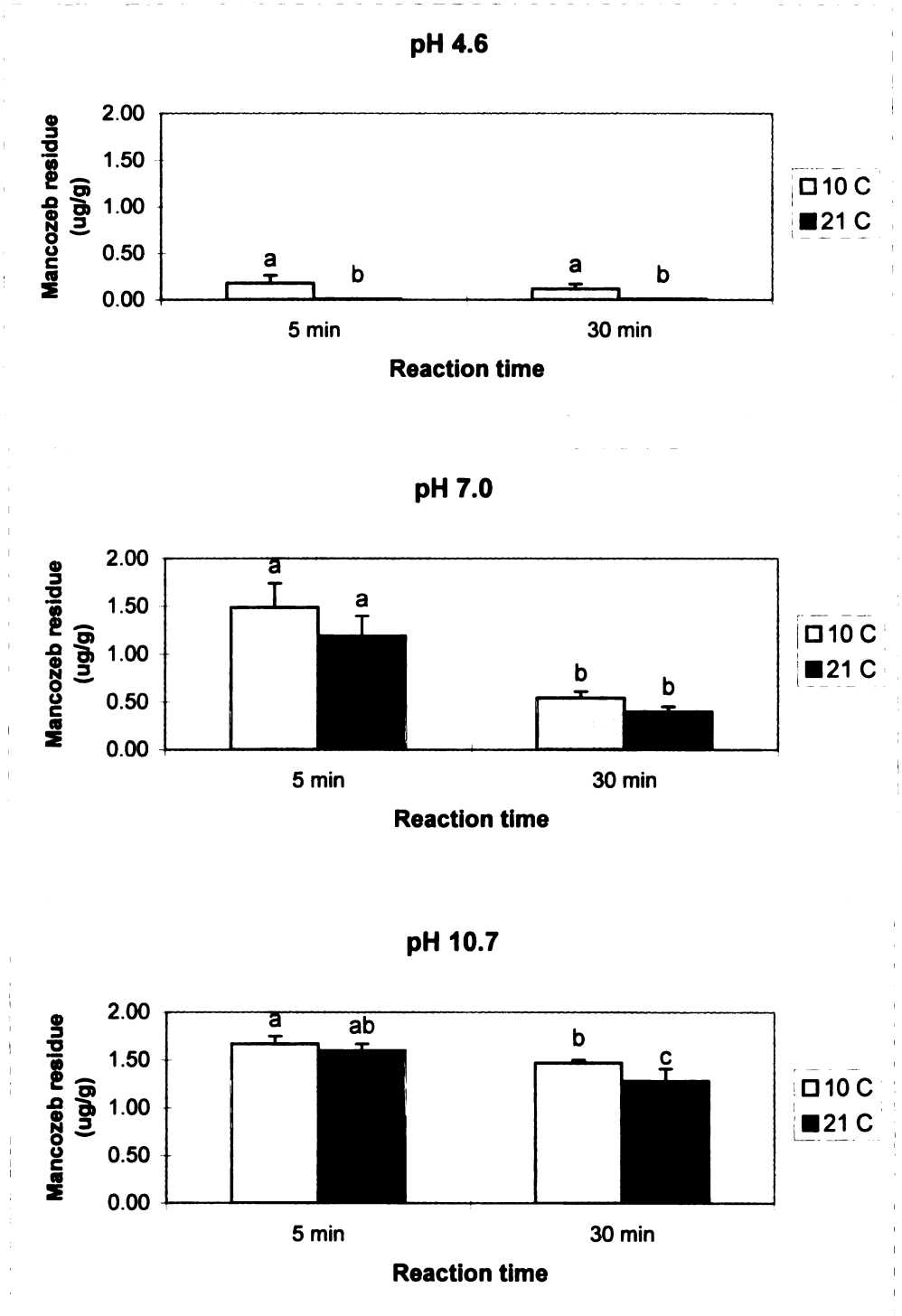


Figure 12. Effects of reaction time and temperature on the degradation of Mancozeb at 50 ppm $Ca(OCl)_2$.

* Values with same letters are not significantly different (p <0.05).

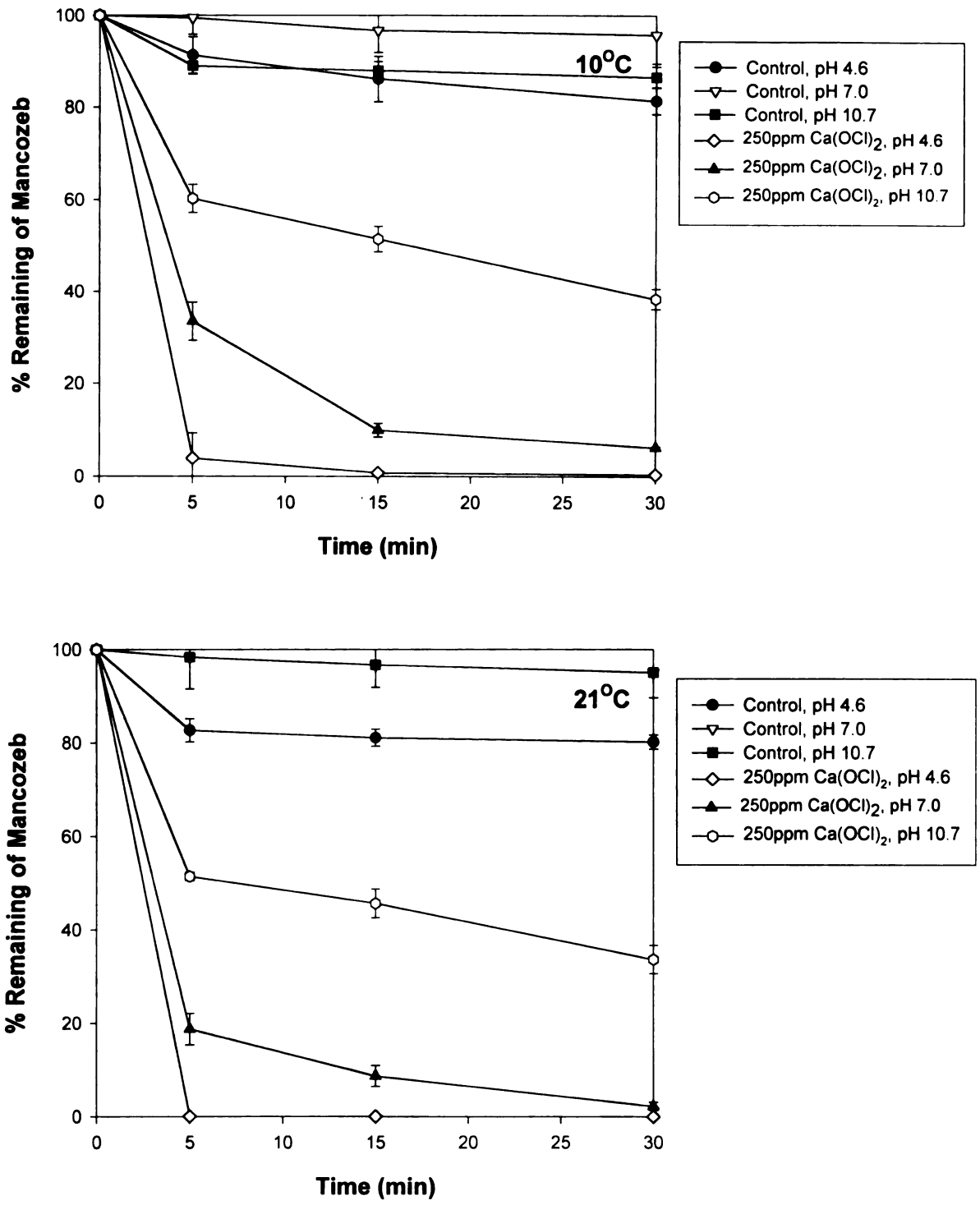


Figure 13. Effect of 250 ppm $Ca(OCl)_2$ on the Degradation of 2 ppm Mancozeb at 10 and 21°C.

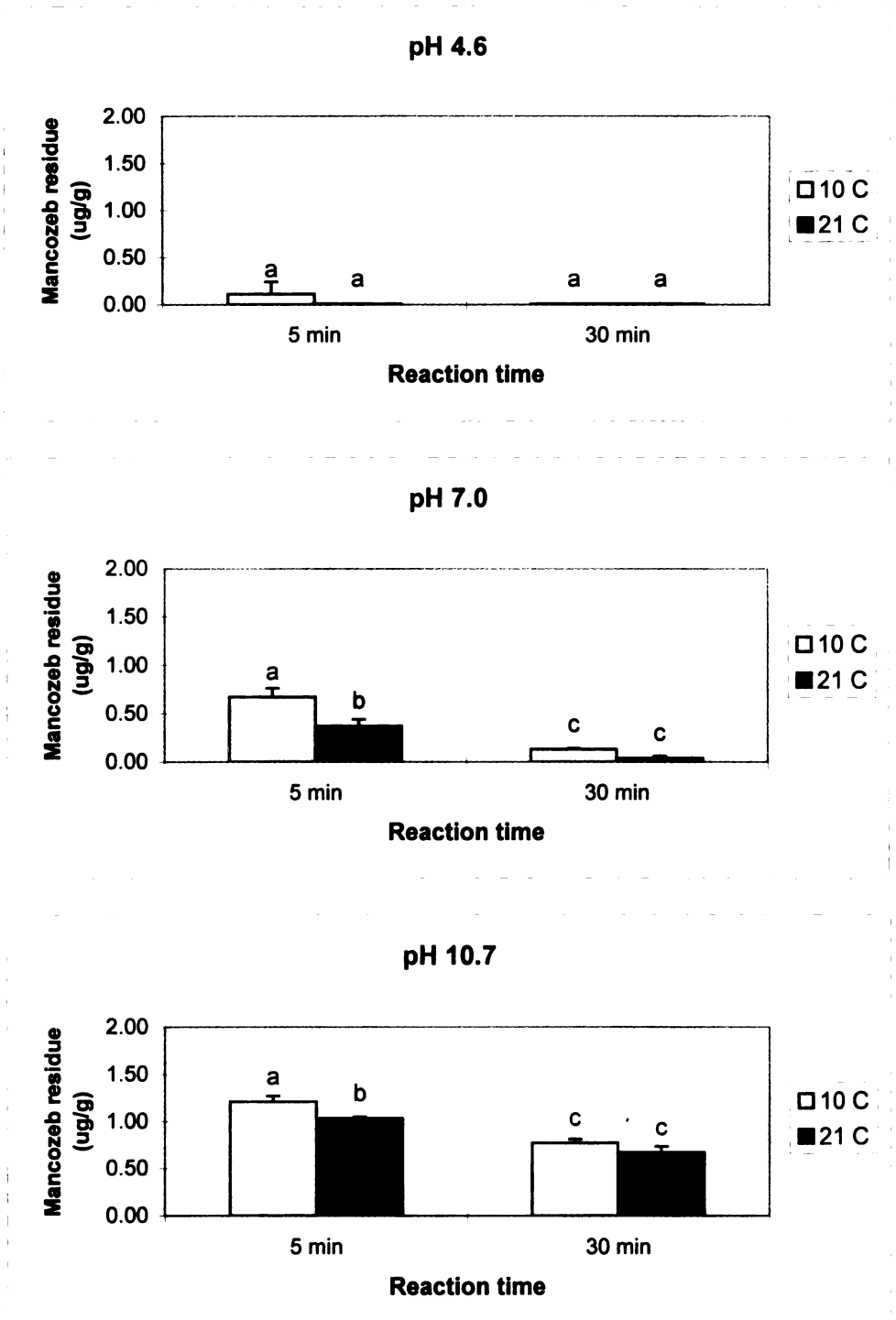


Figure 14. Effects of reaction time and temperature on the degradation of Mancozeb at 250 ppm $Ca(OCl)_2$.

* Values with same letters are not significantly different (p <0.05).

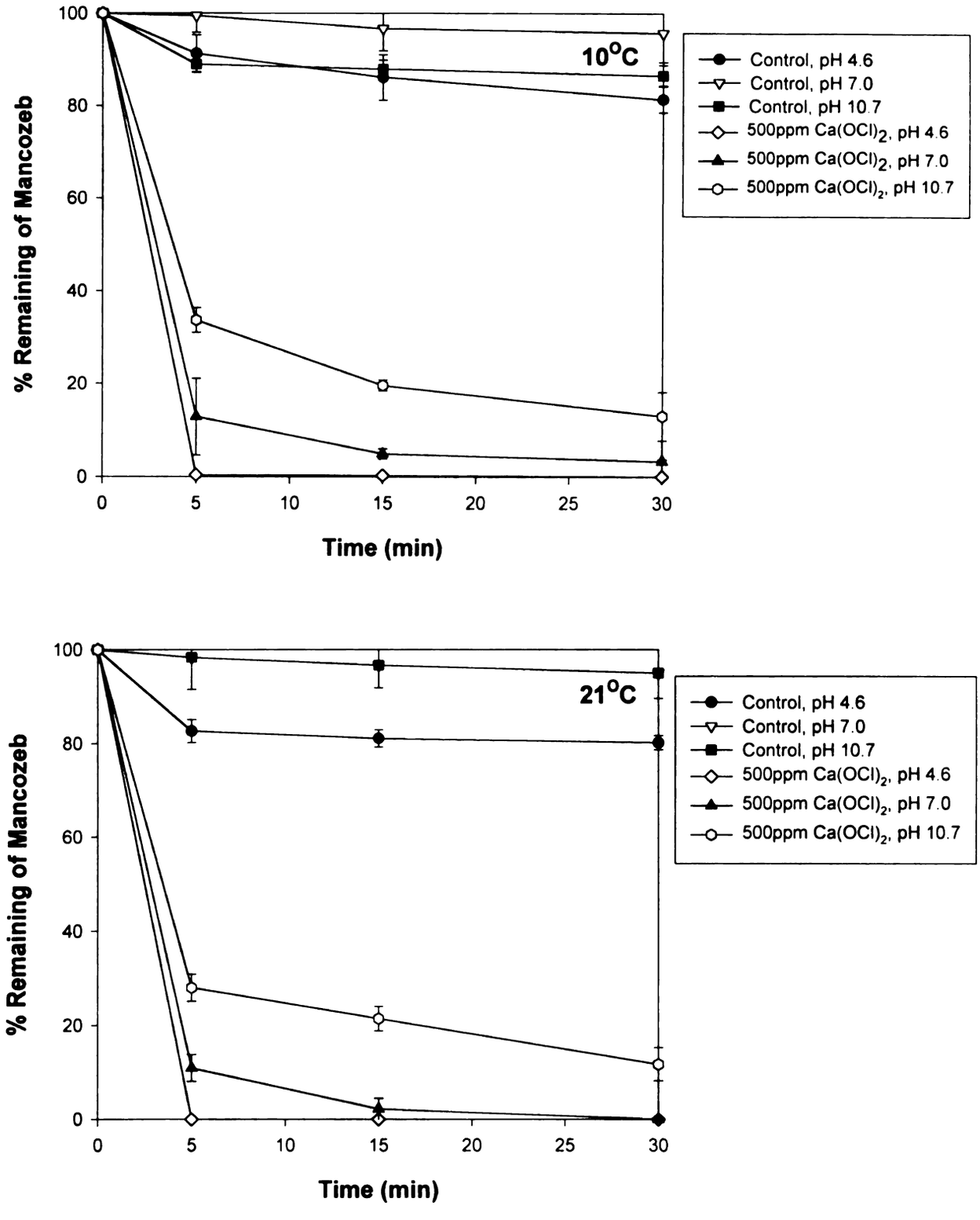


Figure 15. Effect of 500 ppm $Ca(OCl)_2$ on the Degradation of 2 ppm Mancozeb at 10 and 21°C.

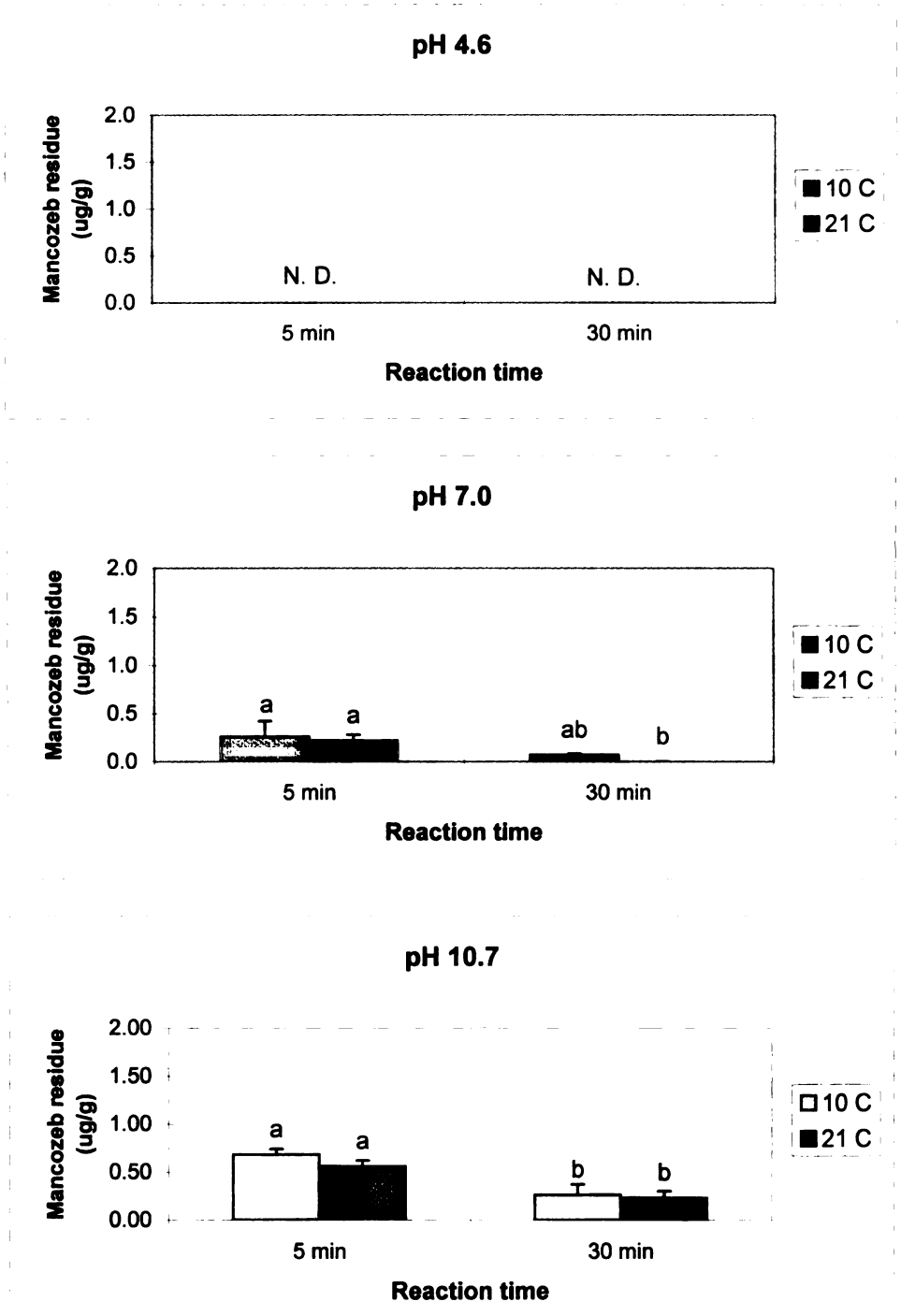


Figure 16. Effects of reaction time and temperature on the degradation of Mancozeb at 500 ppm $Ca(OCl)_2$.

* Values with same letters are not significantly different (p <0.05).

* N. D. = None detected.

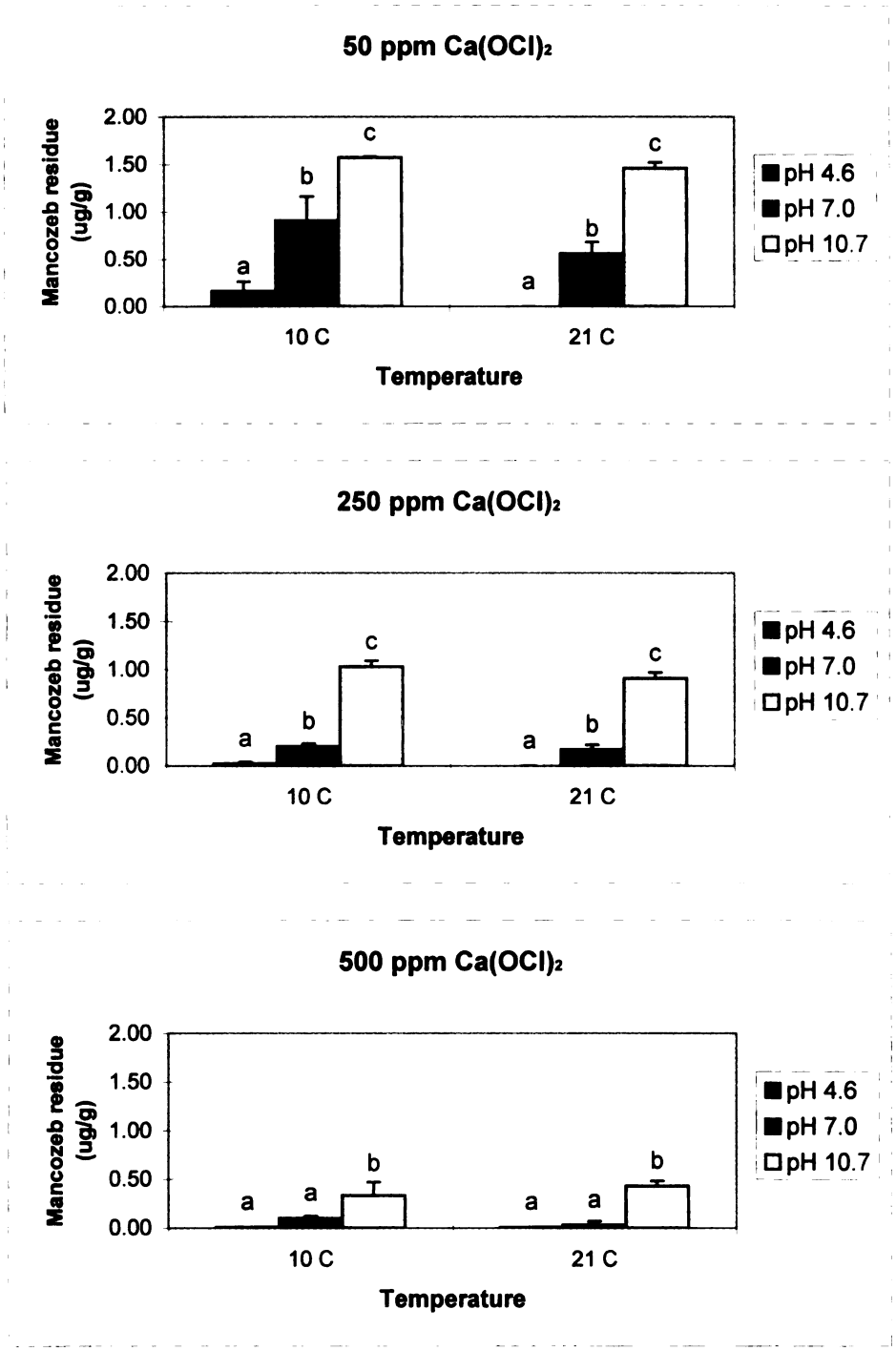


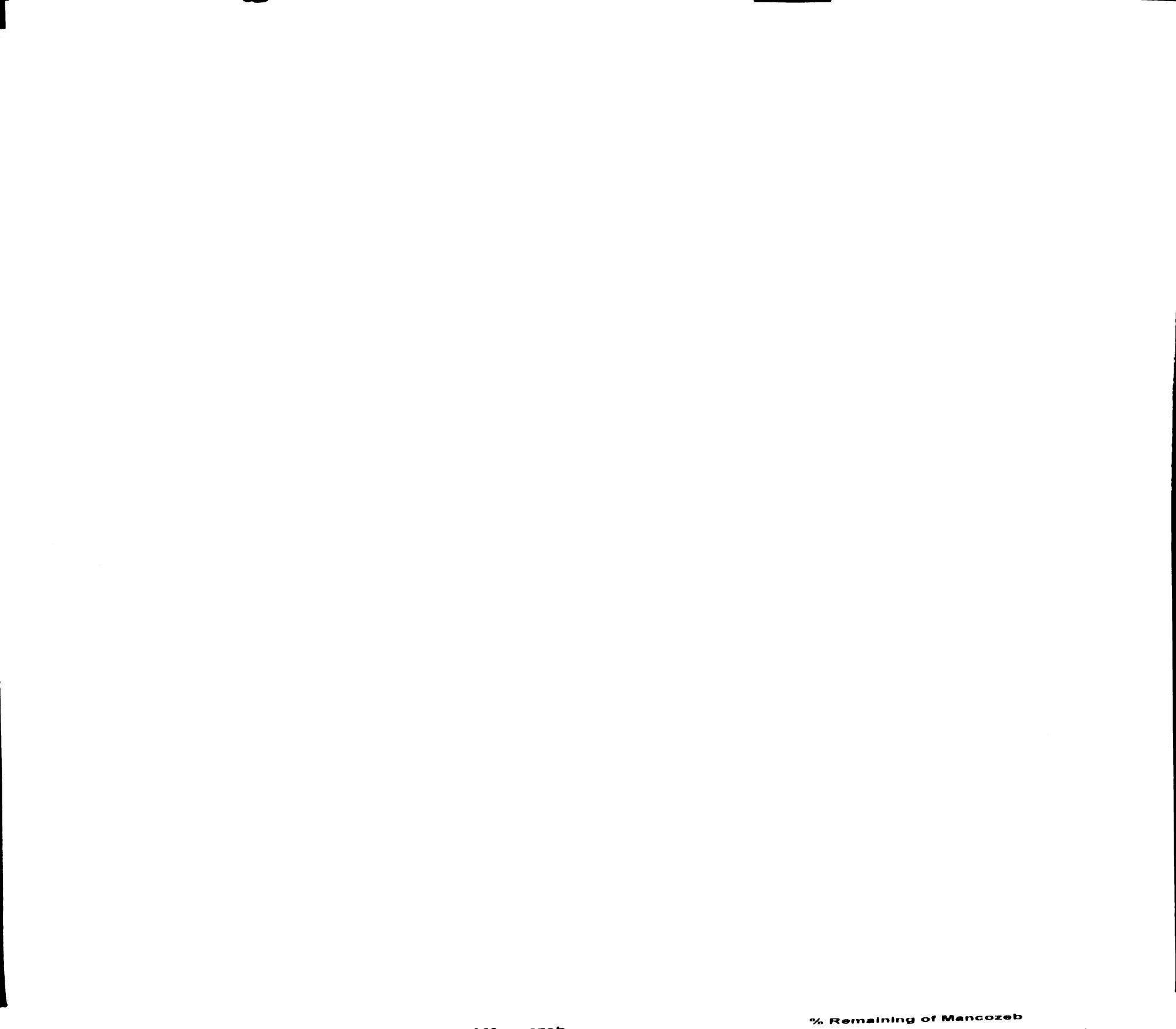
Figure 17. Effects of temperature and pH on the degradation of Mancozeb in $Ca(OCl)_2$ treatments at 15 minute reaction time.

* Values with same letters are not significantly different ($p < 0.05$).

(III) Degradation of Mancozeb by Chlorine Dioxide

Degradation of mancozeb by chlorine dioxide showed a pattern similar to calcium hypochlorite treatment (Figures 18–22). As can be seen in Figure 18 and 20, when chlorine dioxide and liquid chlorine were used to degrade mancozeb residues, the required amount of chlorine dioxide was lower than that of chlorine. Maximum degradation of mancozeb by chlorine dioxide was observed at pH 4.6. For the 5 ppm chlorine dioxide treatment, between 62 and 78% of mancozeb remained after 5 minutes at both 10 and 21°C (Figure 18). Chlorine dioxide at 10 ppm significantly ($p<0.05$) increased the rate of degradation of mancozeb at pH 4.6 at both temperatures. However, there was no significant ($p<0.05$) difference in the degradation of mancozeb between 5 and 10 ppm chlorine dioxide at pH 7.0 and 10.7 at either temperatures. The effects of pH on the degradation of mancozeb in solution are illustrated in Figure 22. The most effective pH for the degradation of mancozeb with chlorine dioxide was in pH 4.6, while pH 10.7 was the least effective treatment.

The mechanism of chlorination and oxidation of organic compounds by chlorine dioxide are not known. Chlorination in aqueous solutions may occur indirectly through a progressive reduction of chlorine dioxide, which passes through the HOCl stage.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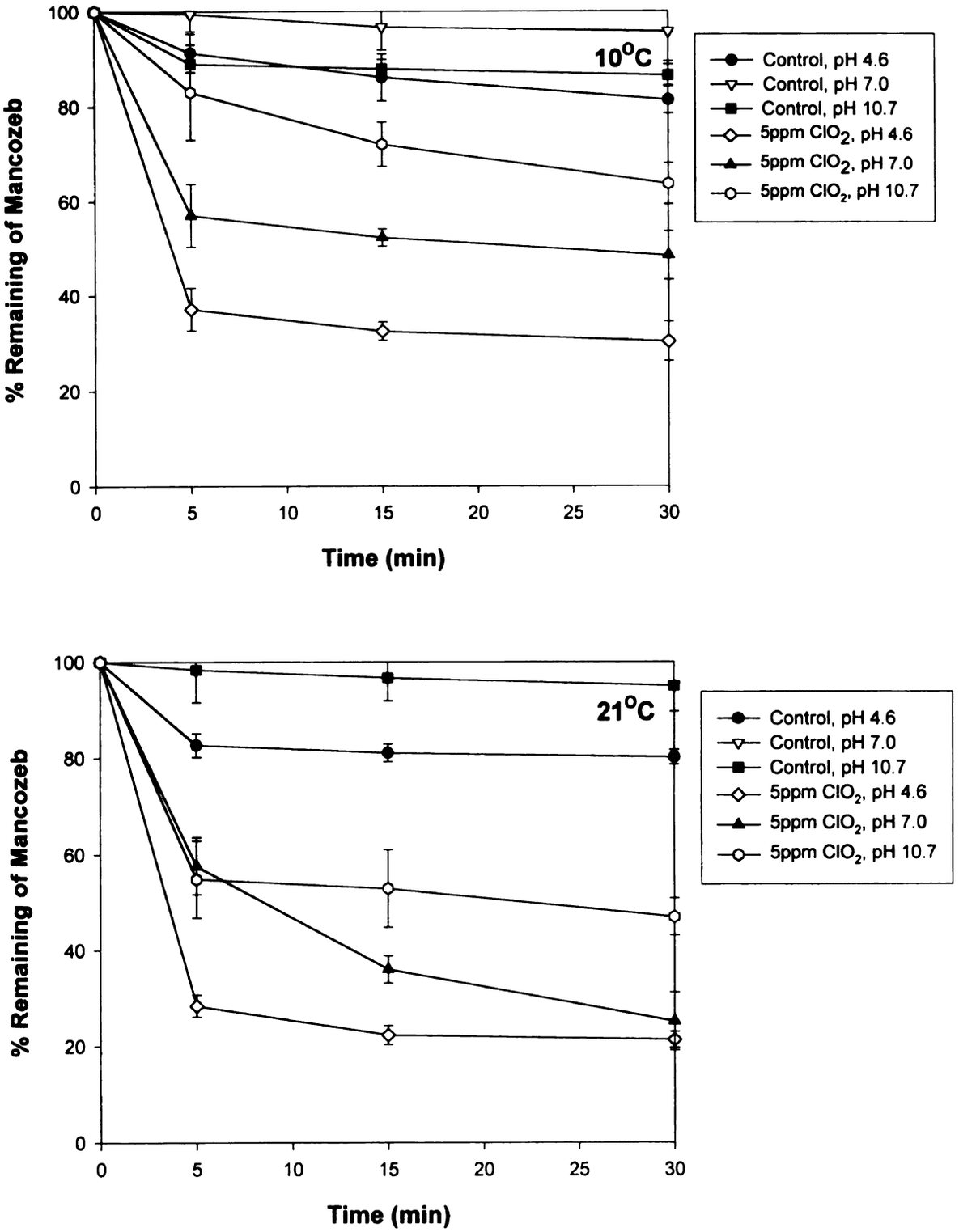

Figure 18. Effect of 5 ppm ClO_2 on the Degradation of 2 ppm Mancozeb at 10 and 21°C.

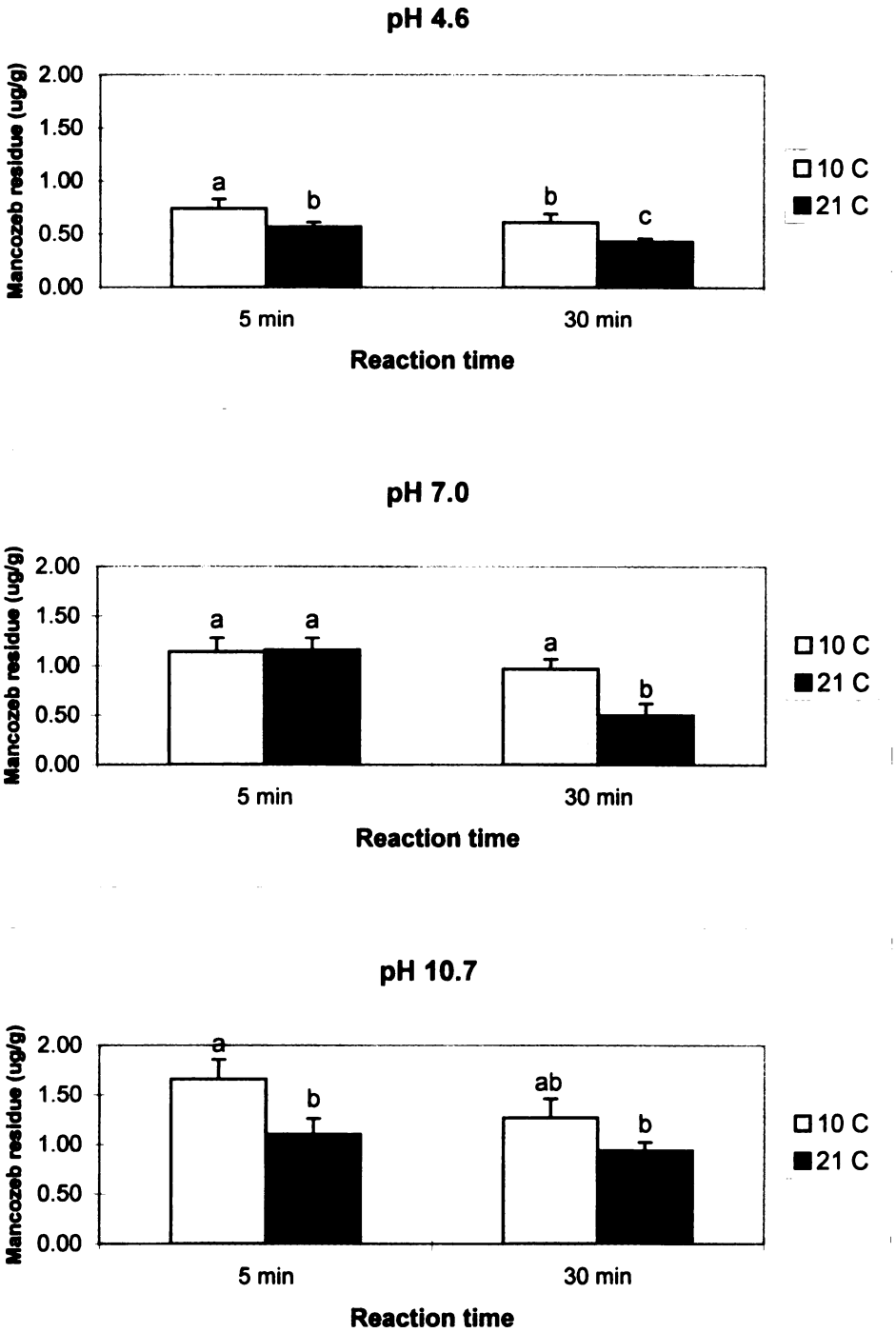

Figure 19. Effects of reaction time and temperature on the degradation of Mancozeb at 5 ppm ClO_2.

* Values with same letters are not significantly different (p <0.05).

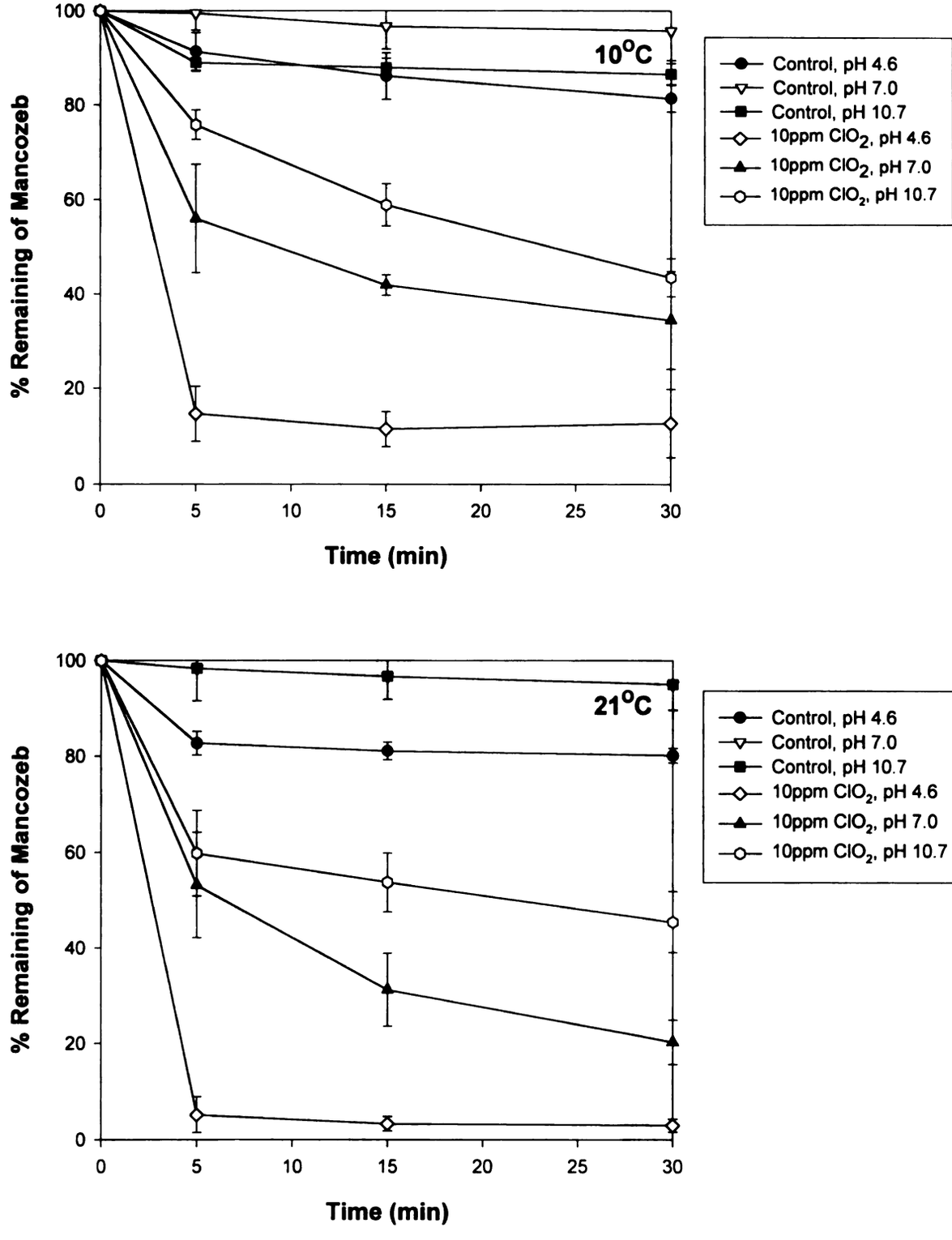

Figure 20. Effect of 10 ppm ClO_2 on the Degradation of 2 ppm Mancozeb at 10 and 21°C.

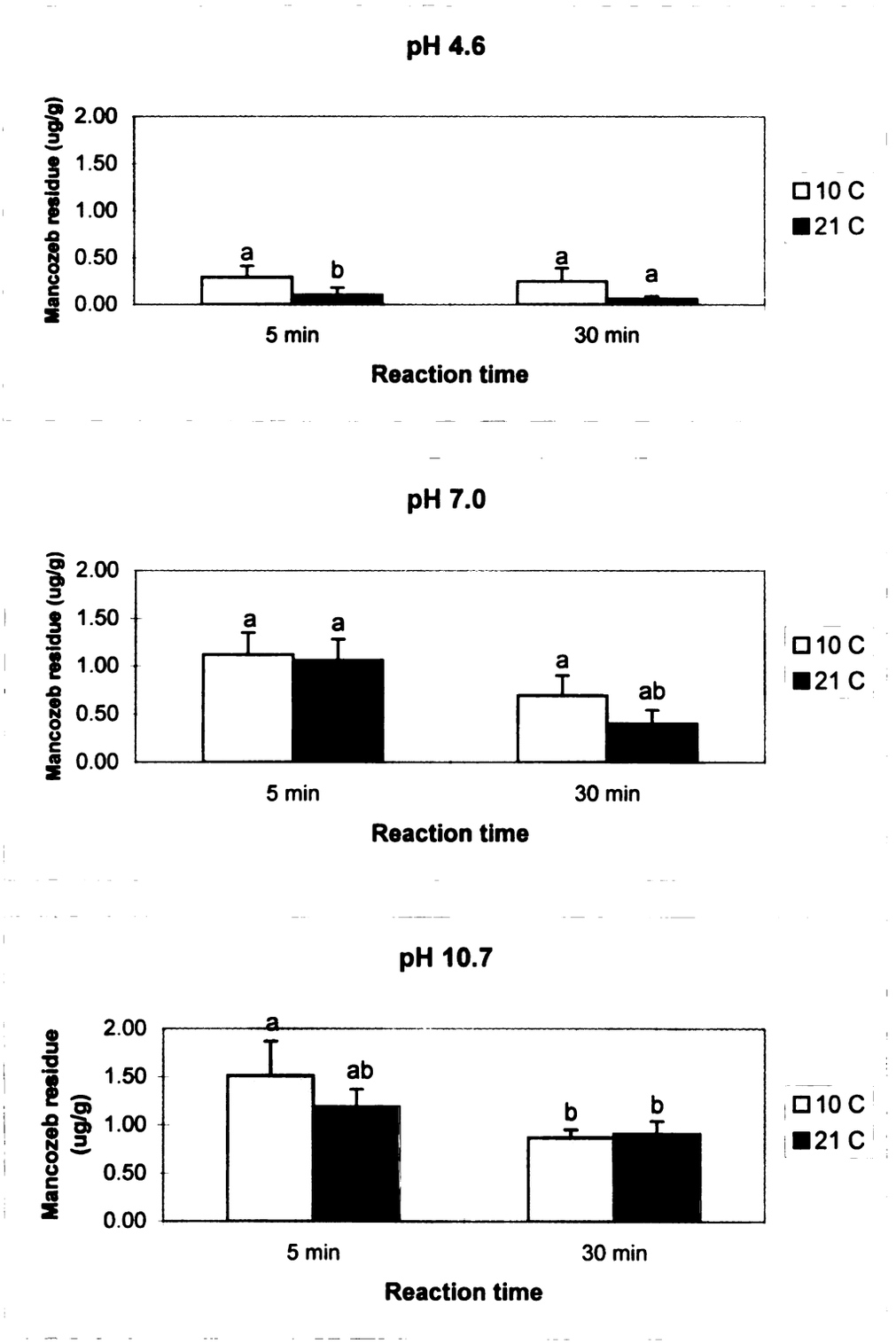

Figure 21. Effects of reaction time and temperature on the degradation of Mancozeb at 10 ppm ClO_2.

* Values with same letters are not significantly different (p <0.05).

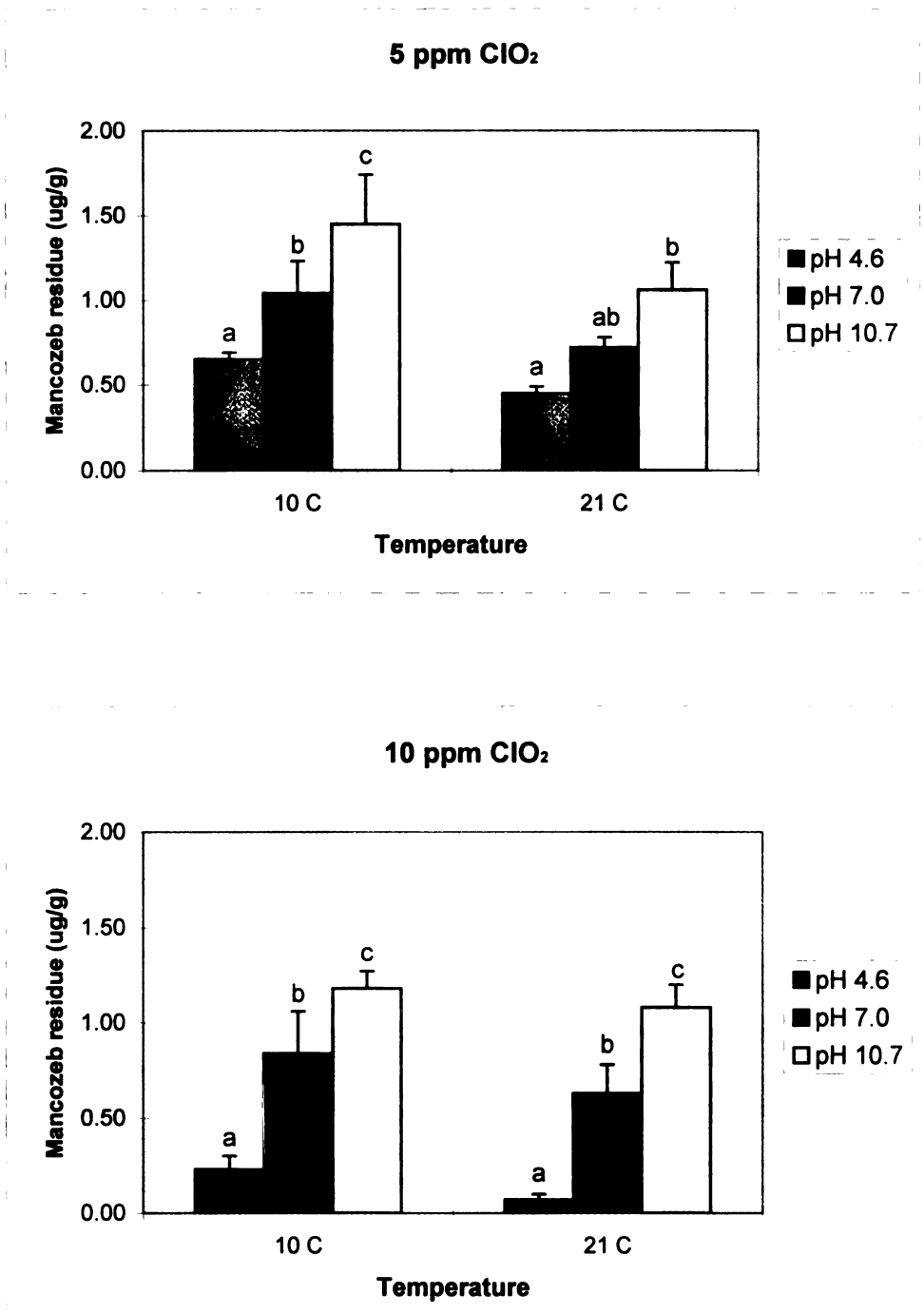

Figure 22. Effects of temperature and pH on the degradation of Mancozeb in ClO_2 treatments at 15 minute reaction time.

* Values with same letters are not significantly different (p <0.05).

(IV) Degradation of Mancozeb by Ozone

Degradation of mancozeb by ozone was greatest at pH 7.0 and decreased with increasing pH (Figures 23–27). The ozone treatment at pH 10.7 was the least effective at both 10°C and 21°C. Its degradation was only 10% and 18% after 5 and 30 minutes, respectively at 21°C (Figures 23, 25). For the 1 ppm ozone treatment, almost 96% of the initial amount of mancozeb was degraded after 30 minutes at pH 7.0 and ambient temperature (Figure 23). Ozonation at 3 ppm significantly ($p<0.05$) increased the rate of degradation of mancozeb in pH 4.6 and pH 7.0 treatments at ambient temperature. Only about 1% of mancozeb remained at pH 7.0 after 30 minutes at 21°C. At pH 7.0, almost 65% of the initial amount of mancozeb was degraded after only 5 minutes in a 3 ppm ozone concentration (Figure 25). Ozone degraded the majority of the mancozeb residues within the first 5 minutes. This has important implications from a practical situation, since the time required to lower the concentration of any pesticide will affect cost. Again, the most effective treatment was ozonation at 3 ppm in the pH 7.0 solution, while pH 10.7 was the least effective treatment (Figure 27).

Many factors govern the solubility of ozone in water, one being temperature. Ozone is partially soluble in water and, like most gases, increases in solubility as the water temperature decreases. Dissolved

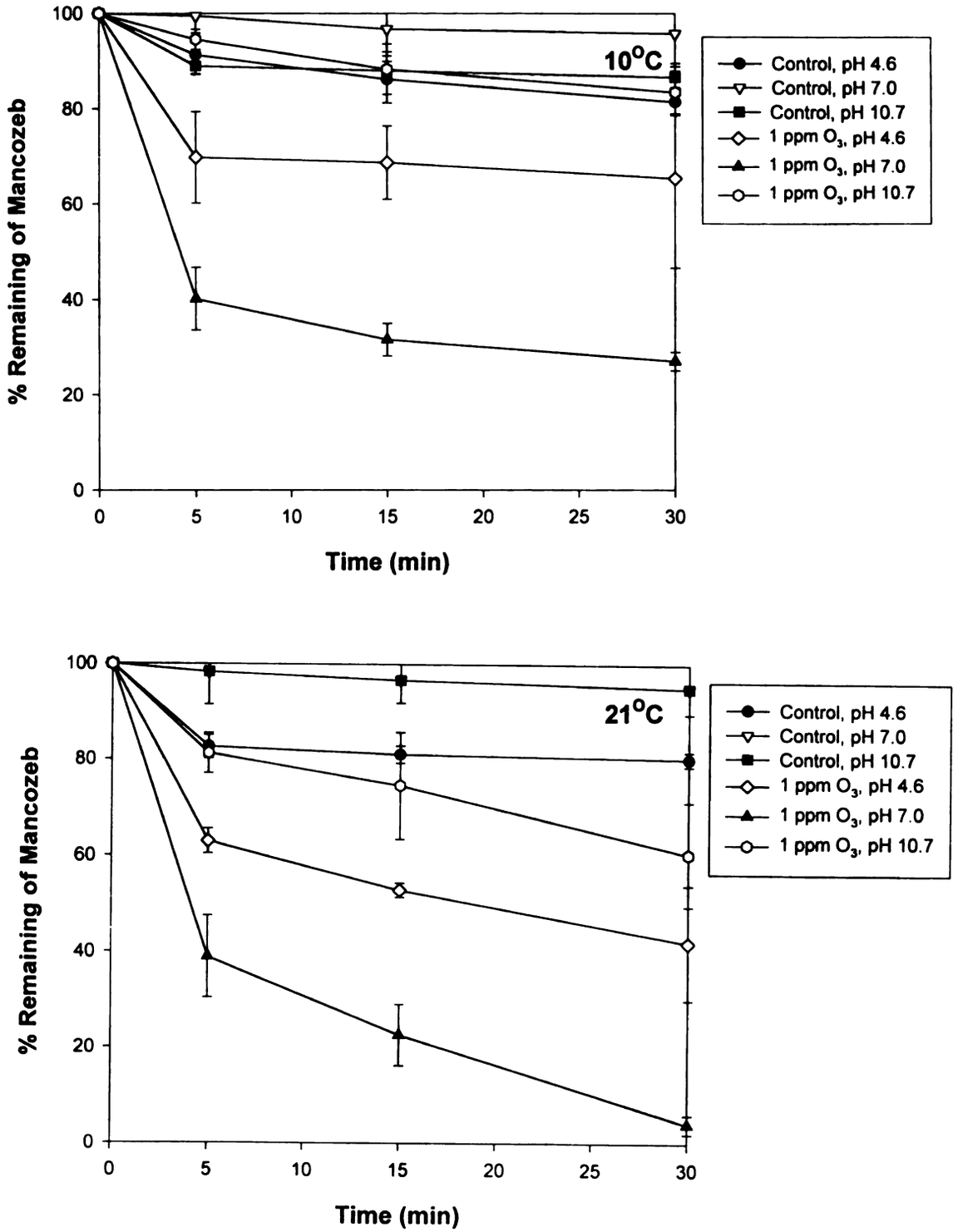

Figure 23. Effect of 1 ppm O_3 on the degradation of 2 ppm Mancozeb at 10 and 21°C.

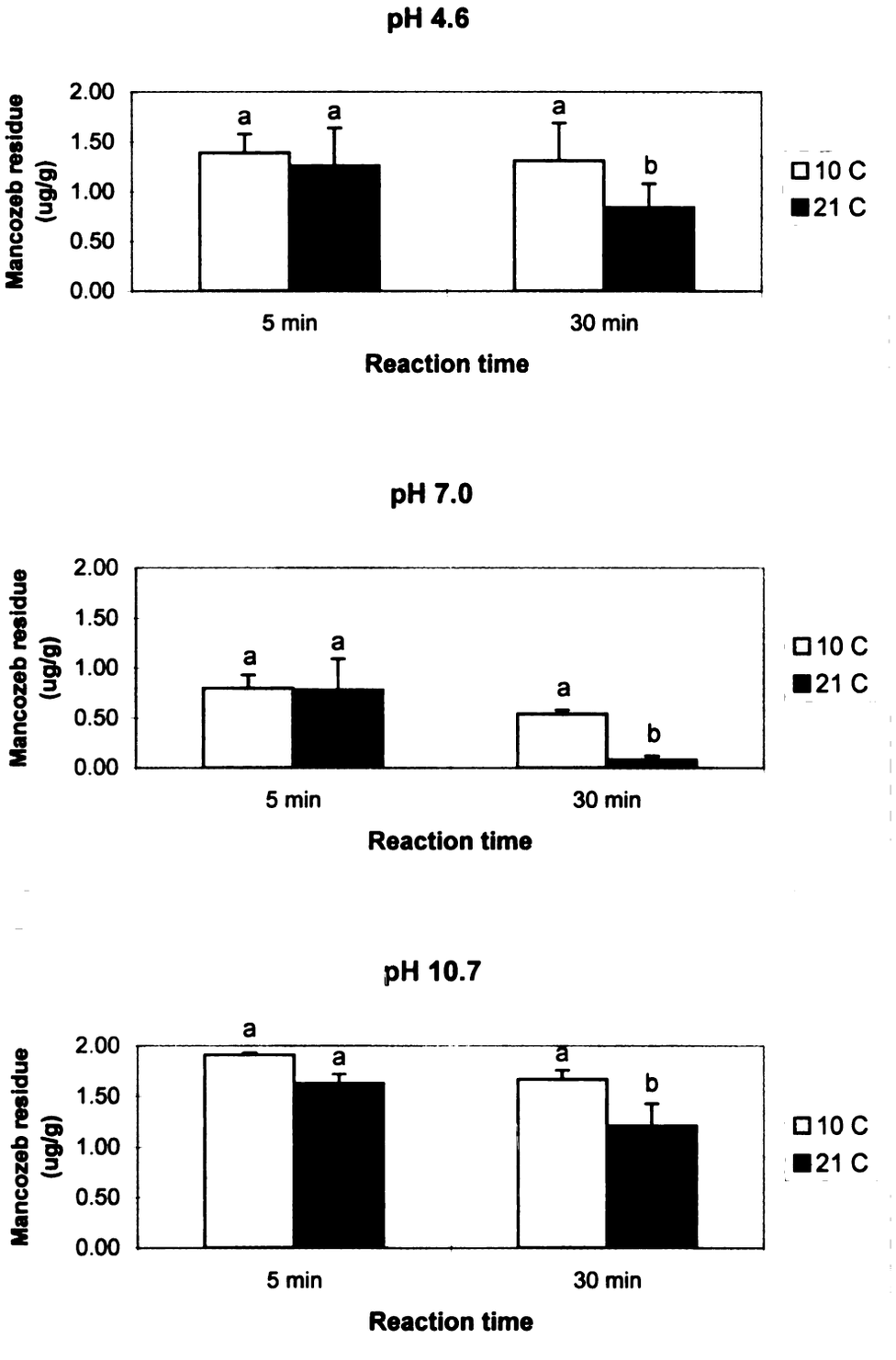

Figure 24. Effects of reaction time and temperature on the degradation of Mancozeb at 1 ppm O_3.

* Values with same letters are not significantly different (p <0.05).

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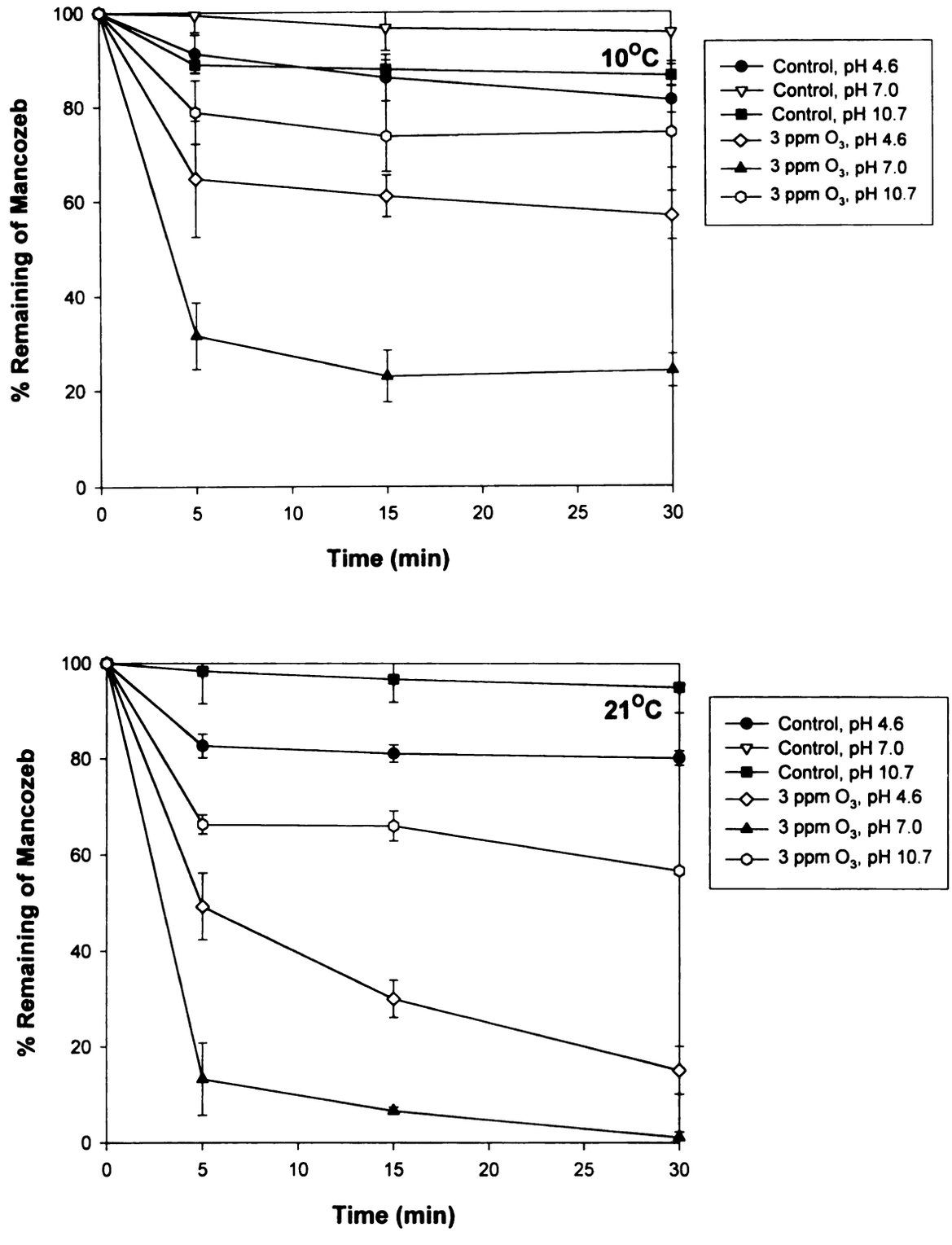


Figure 25. Effect of 3 ppm O_3 on the degradation of 2 ppm Mancozeb at 10 and 21°C.

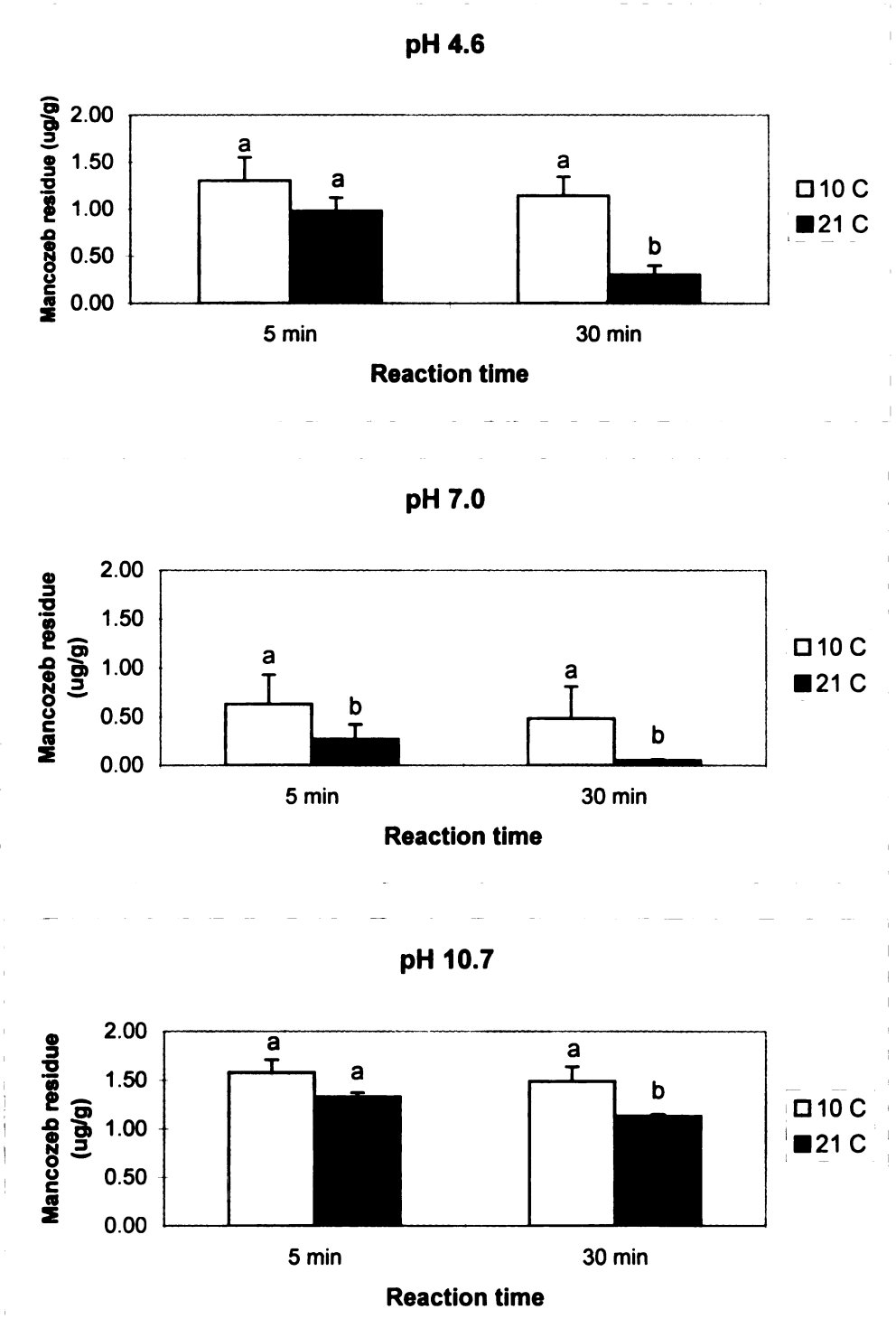


Figure 26. Effects of reaction time and temperature on the degradation of Mancozeb at 3 ppm O_3.

* Values with same letters are not significantly different (p <0.05).

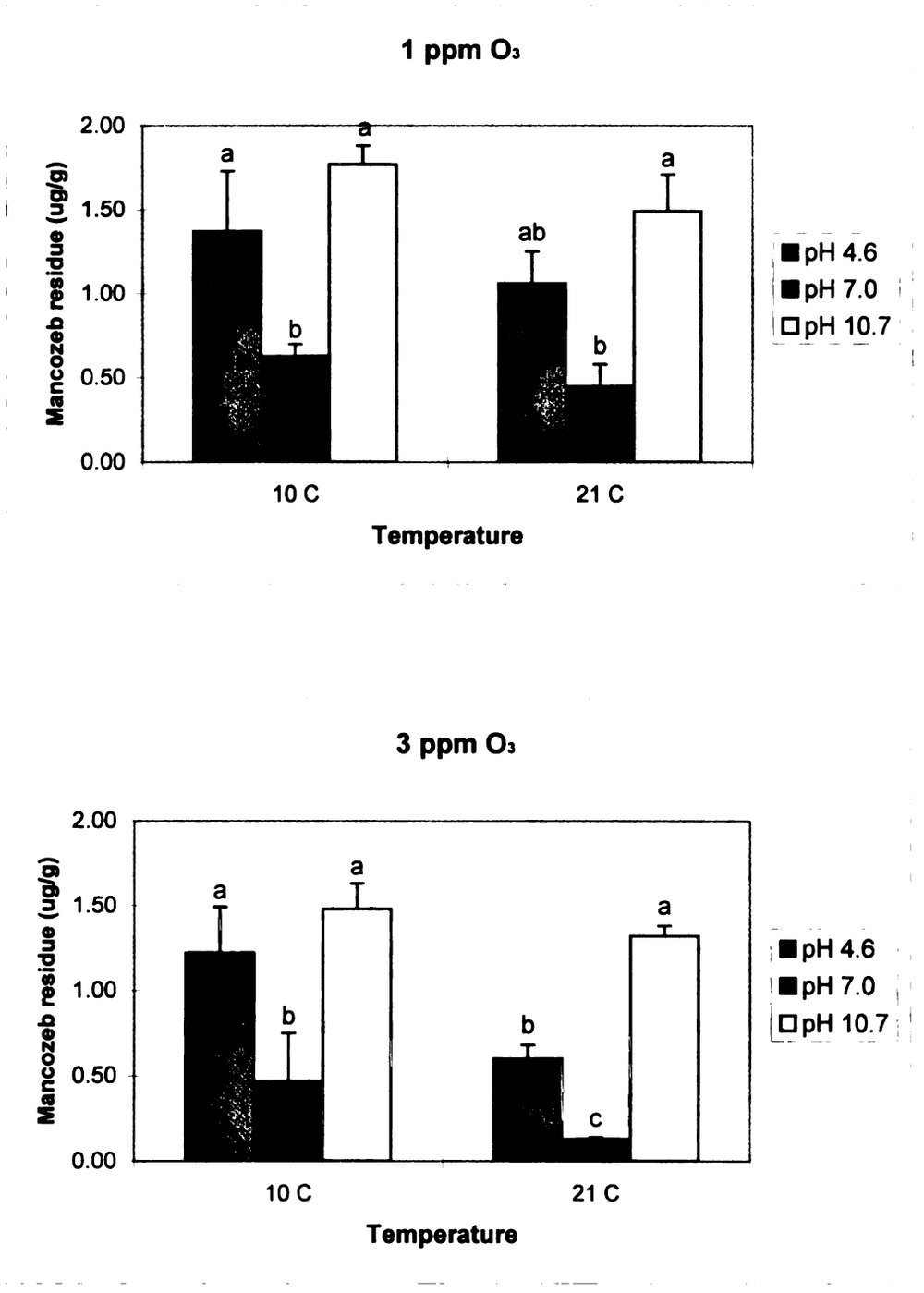


Figure 27. Effects of temperature and pH on the degradation of Mancozeb in O_3 treatments at 15 minute reaction time.

* Values with same letters are not significantly different (p <0.05).

ozone residuals also decrease with increasing temperature, due to thermal decomposition (Hewes and Davison, 1971), which could adversely effect the overall degradation process. In this study, two temperatures, 10°C and 21°C, were used.

Ozone has the unique property of autodecomposition, producing numerous free radical species, the most prominent being the hydroxyl free radical (OH•). As the pH of solutions containing dissolved ozone increases, the rate of decomposition of molecular ozone to produce hydroxyl free radicals also increases, such that at a pH of about 10, ozone decomposes instantaneously (Graham, 1997). Kearney *et. al.* (1988) found that ozonation at high pH was less effective, due to the instability of ozone in solution as the pH increases. This is due to the catalytic effect of hydroxyl ions on the ozone decomposition process. As the hydroxide ion is a promoter of ozone decomposition, the half-life of ozone is very short under alkaline conditions. At pH 10, the half-life for ozone in pure water is approximately 30 seconds (Masten *et al.*, 1994). Therefore, pH increases reduced the effect of ozone on the degradation of mancozeb, while the effect of hydrolysis increased slightly.

(V) Degradation of Mancozeb by Hydrogen Peroxyacetic Acid

Maximum degradation of mancozeb by HPAA was observed at pH 7.0 (Figures 28–32). For the 5 ppm HPAA treatment, between 50 and

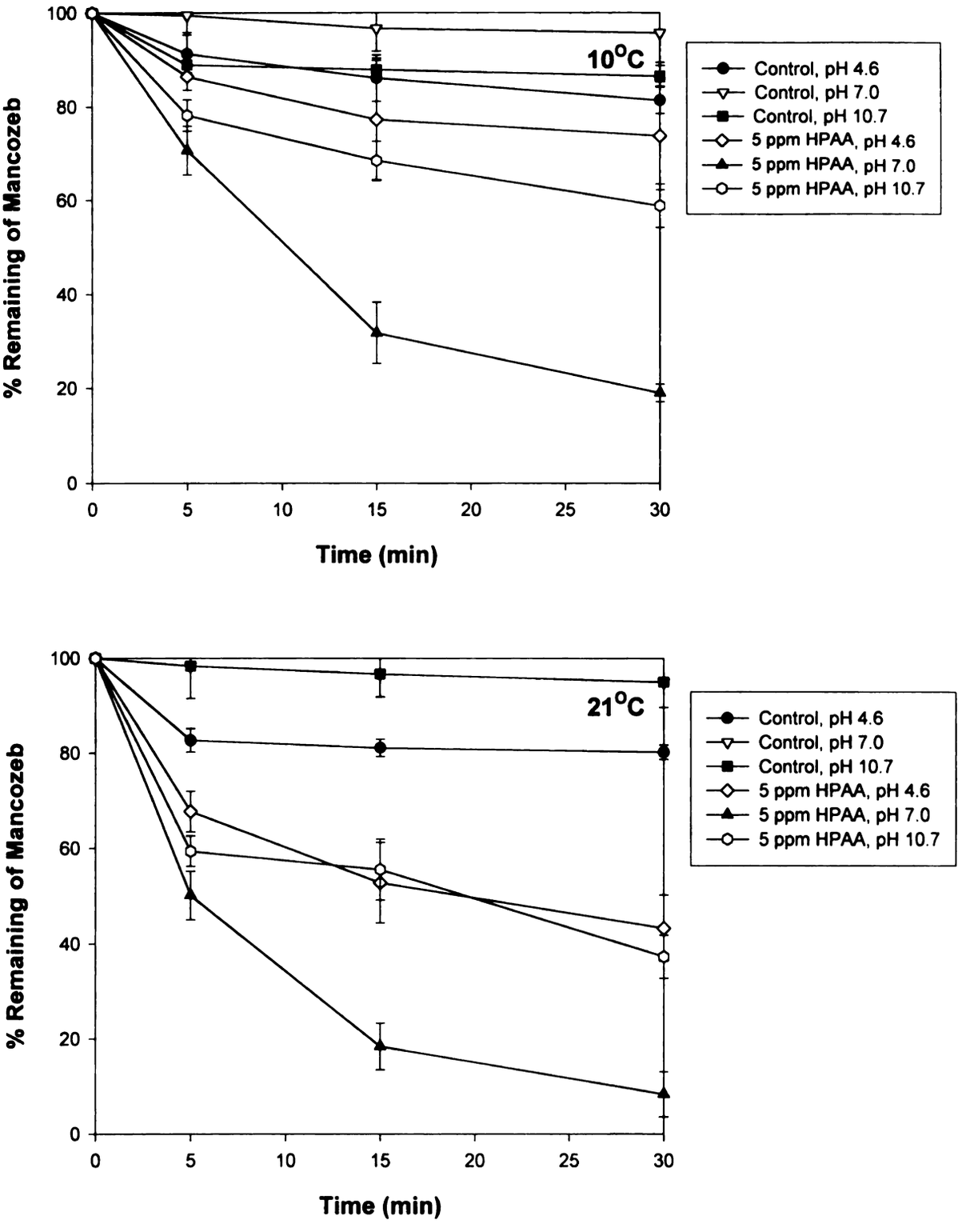


Figure 28. Effect of 5 ppm HPAA on the degradation of 2 ppm Mancozeb at 10 and 21°C.

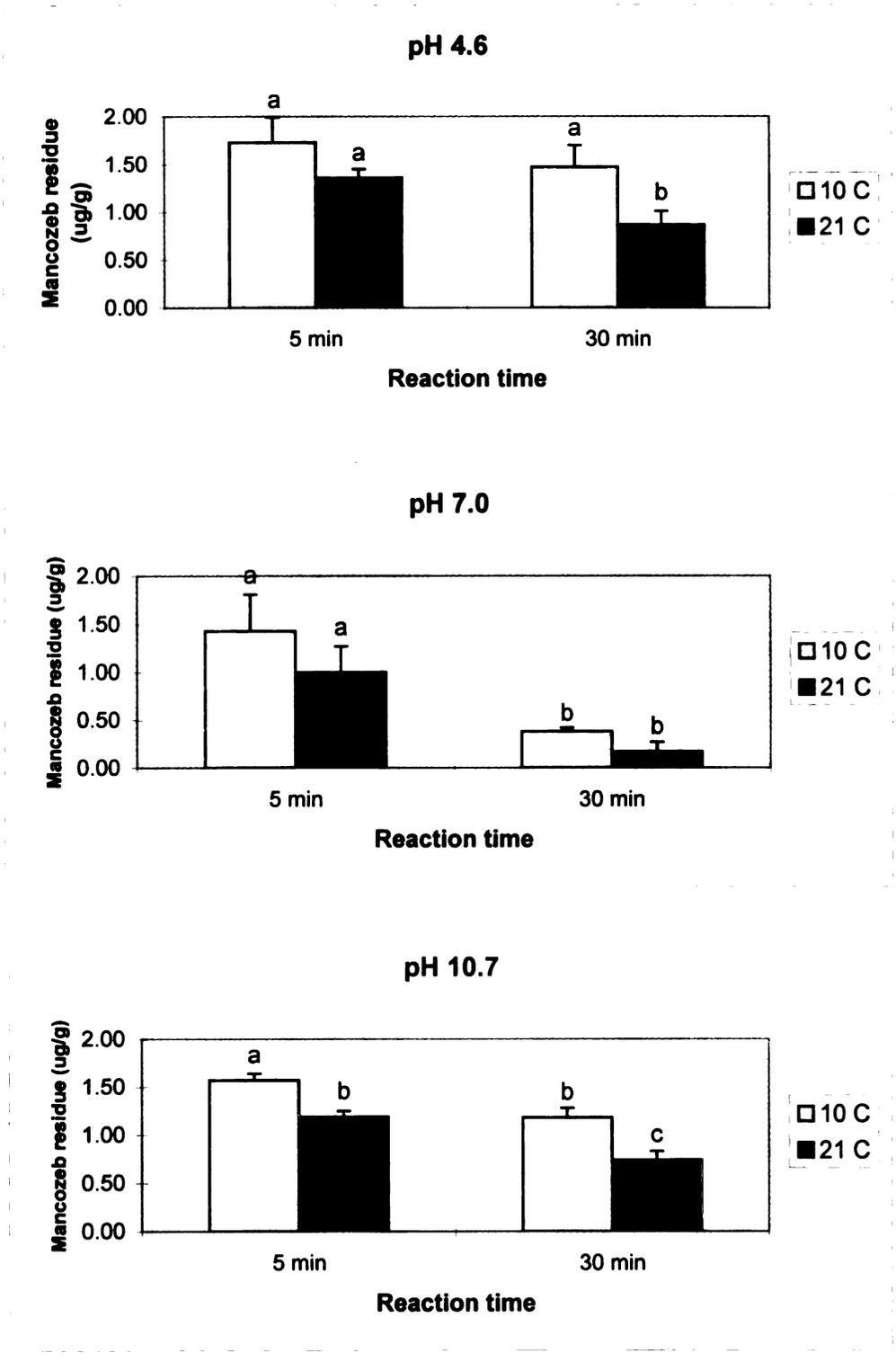


Figure 29. Effects of reaction time and temperature on the degradation of Mancozeb at 5 ppm HPAA.

* Values with same letters are not significantly different (p <0.05).

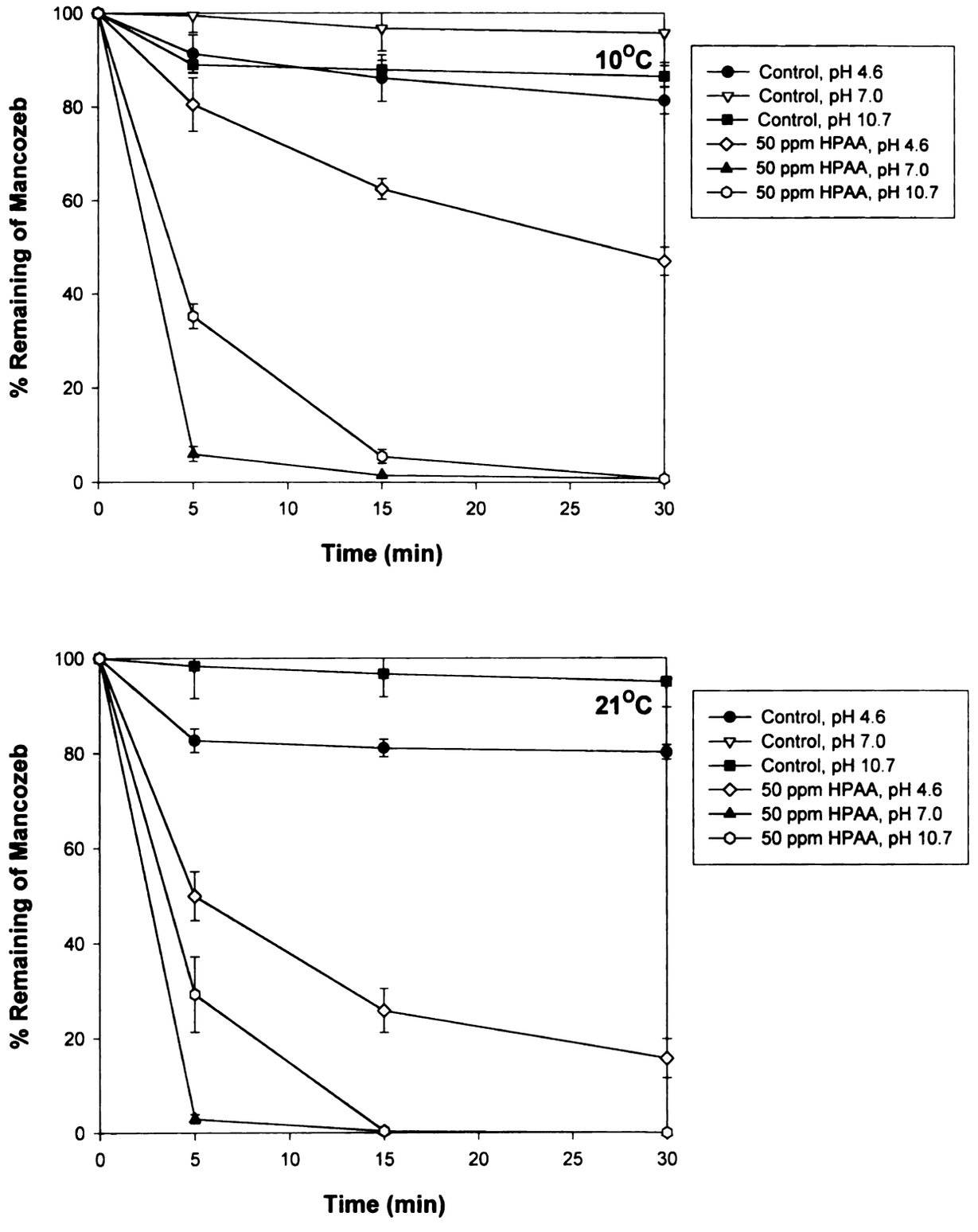


Figure 30. Effect of 50 ppm HPAA on the degradation of 2 ppm Mancozeb at 10 and 21°C.

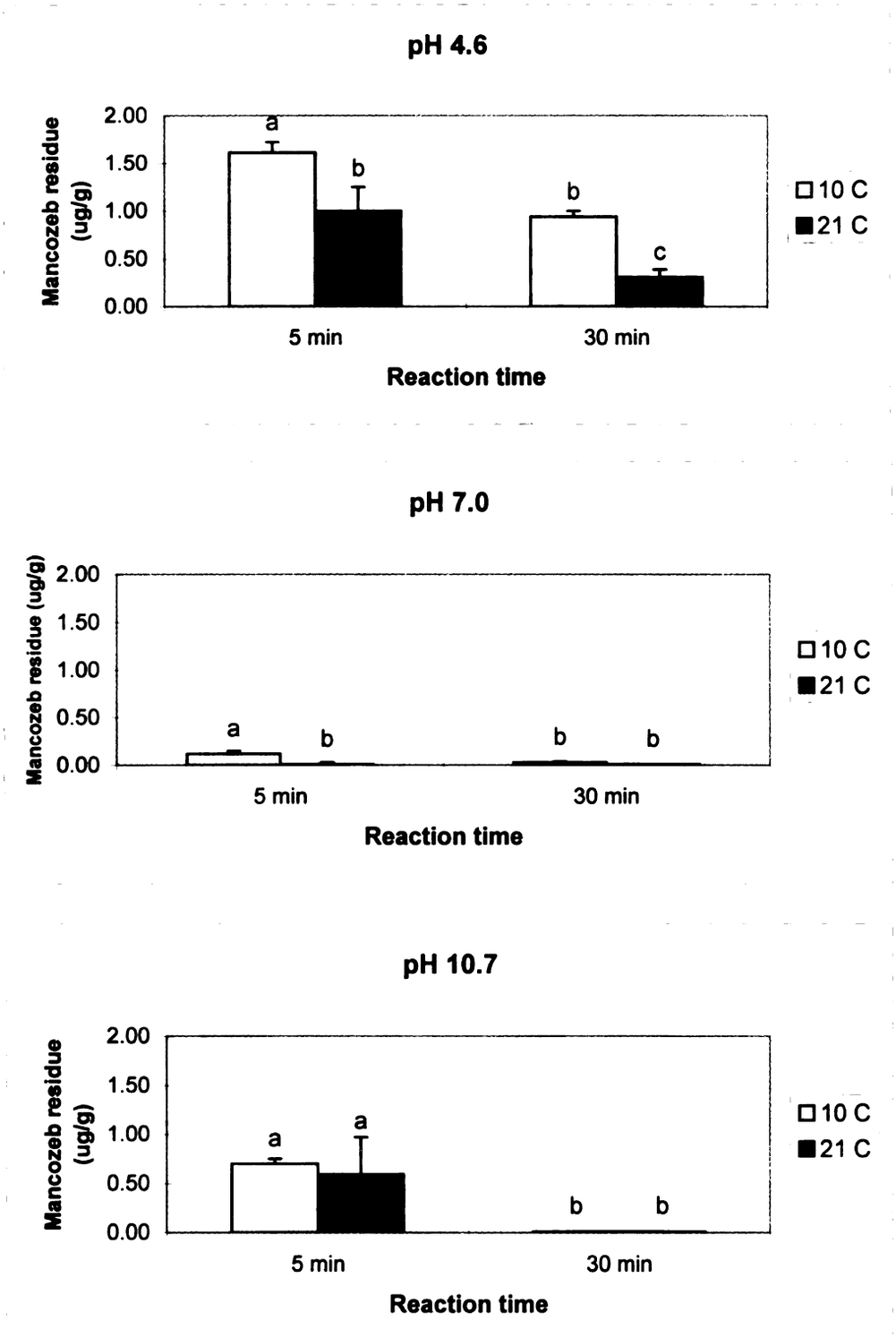


Figure 31. Effects of reaction time and temperature on the degradation of Mancozeb at 50 ppm HPAA.

* Values with same letters are not significantly different (p <0.05).

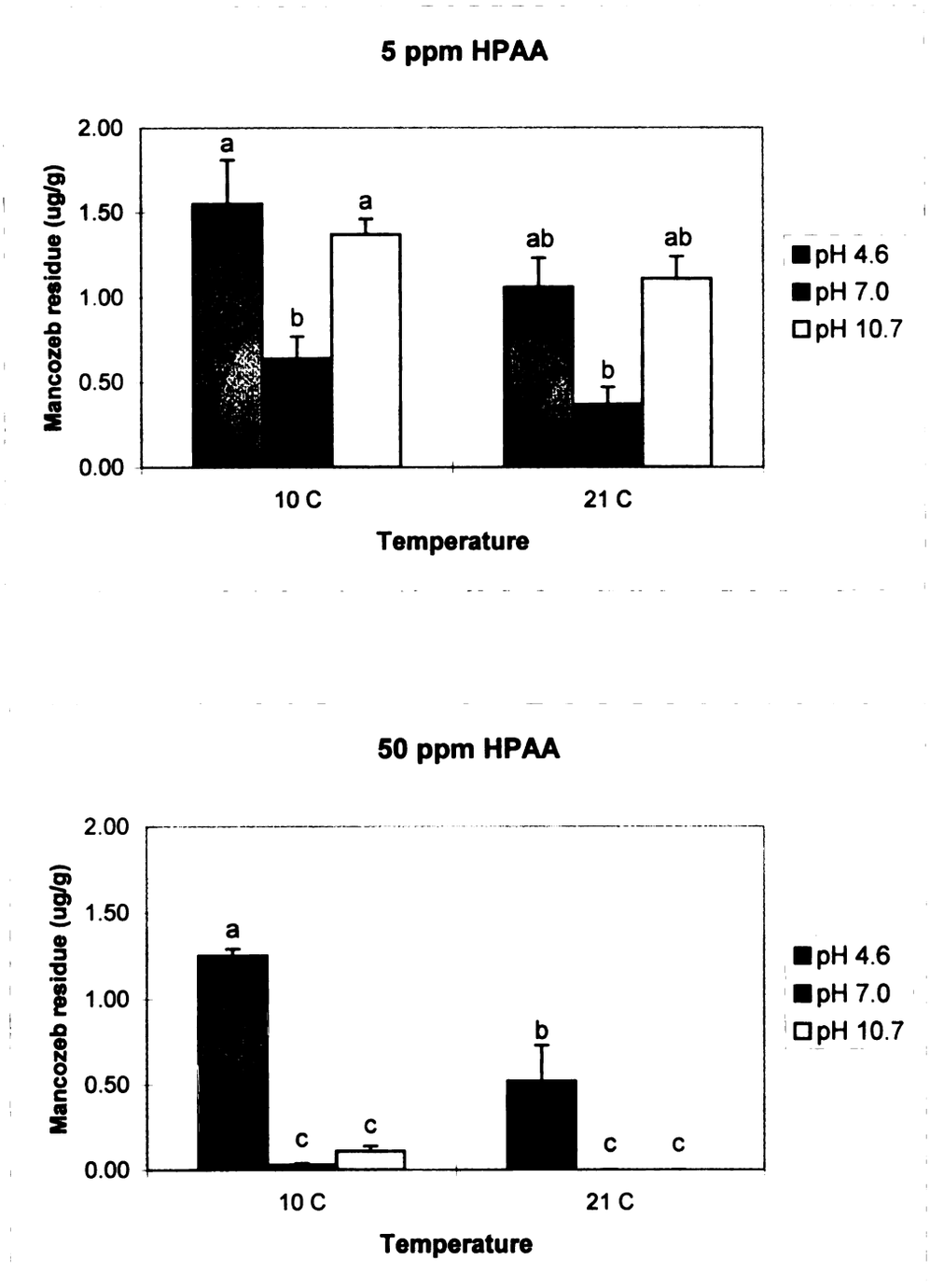


Figure 32. Effects of temperature and pH on the degradation of Mancozeb in HPAA treatments at 15 minute reaction time.

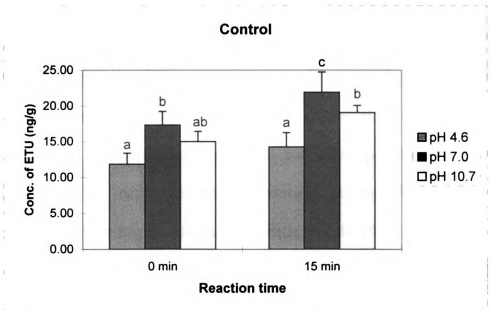
* Values with same letters are not significantly different (p <0.05).

70% of mancozeb remained after 5 minutes at both 10 and 21°C at pH 7.0. Treatments at pH 4.6 and pH 10.7 were less effective than pH 7.0. Degradation of mancozeb at pH 7.0 at both 10 and 21°C was significantly (p<0.05) different than at pH 4.6 (Figures 28–29). The HPAA treatment at pH 4.6 was the least effective at both 10 and 21°C with 45–75% degradation after 30 minutes. The 50 ppm HPAA treatment for the degradation of mancozeb was much more effective than 5 ppm HPAA for all three pH treatments and at both temperatures. Increased temperature completely degraded mancozeb after 15 minutes in 50 ppm HPAA at 21°C (Figures 30–31). HPAA treatment at neutral pH was more effective than alkaline or acidic conditions (Figure 32). This relates to the stability of HPAA at various pH ranges.

C. Degradation of ETU in Solution

(I) Degradation of ETU by Hydrolysis

The degradation of mancozeb to ETU in solution due to hydrolysis shown in Figure 33. It was found that the rate of decomposition of mancozeb to ETU was influenced by pH. The total yield of ETU was decreased when the pH was lowered from 7.0 or 10.7 to 4.6. At pH 7.0, the initial ETU concentration was 17.3 ppb, which increased to 21.9 ppb after 15 minutes and then decreased to 12.3 ppb after 60 minutes. In the case of pH 4.6, the initial ETU concentration was 11.9



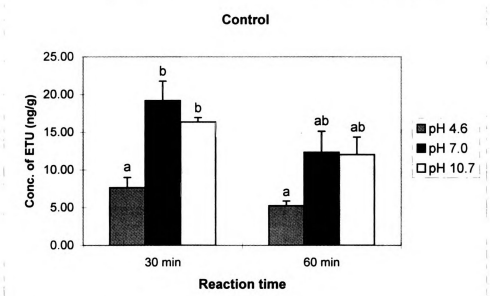


Figure 33. Effects of pH and reaction time on the conversion of Mancozeb into ETU in control.

* Values with same letters are not significantly different (p <0.05).

ppb, which increased to 14.3 ppb after 15 minutes and then decreased to 5.3 ppb after 60 minutes. This shows that acidic pH is much more effective in reducing the conversion rate of mancozeb into ETU compared with neutral or alkaline pH ranges. In processing, acidic treatments can be used as a preventative method for ETU production. Engst and Schnaak (1974) reported that ethylenebisdithiocarbamic acid readily forms ETU under highly alkaline conditions (pH 10.5). As shown in figure 34–41, conversion of mancozeb to ETU reached a maximum at 15 minute reaction time and then decreased for all three pH ranges and all treatments. Appendix 4 shows raw data for ETU residues from the degradation of mancozeb in a model system.

(II) Degradation of ETU by Calcium Hypochlorite

Degradation of ETU by calcium hypochlorite solution was greatest at pH 4.6 and decreased with increasing pH (Figures 34–35). The chlorine treatment at pH 10.7 was the least effective at both 50 and 250 ppm. Its degradation was only about 89 and 75% after 5 and 15 minutes, respectively at 50 ppm calcium hypochlorite (Figure 34). In 50 ppm calcium hypochlorite solution, ETU was completely degraded at pH 4.6 after 5 minutes at ambient temperature. Longer reaction time and higher chlorine concentration increased the degradation of ETU at both 50 and 250 ppm (Figure 35). Chlorination at 50 and 250 ppm

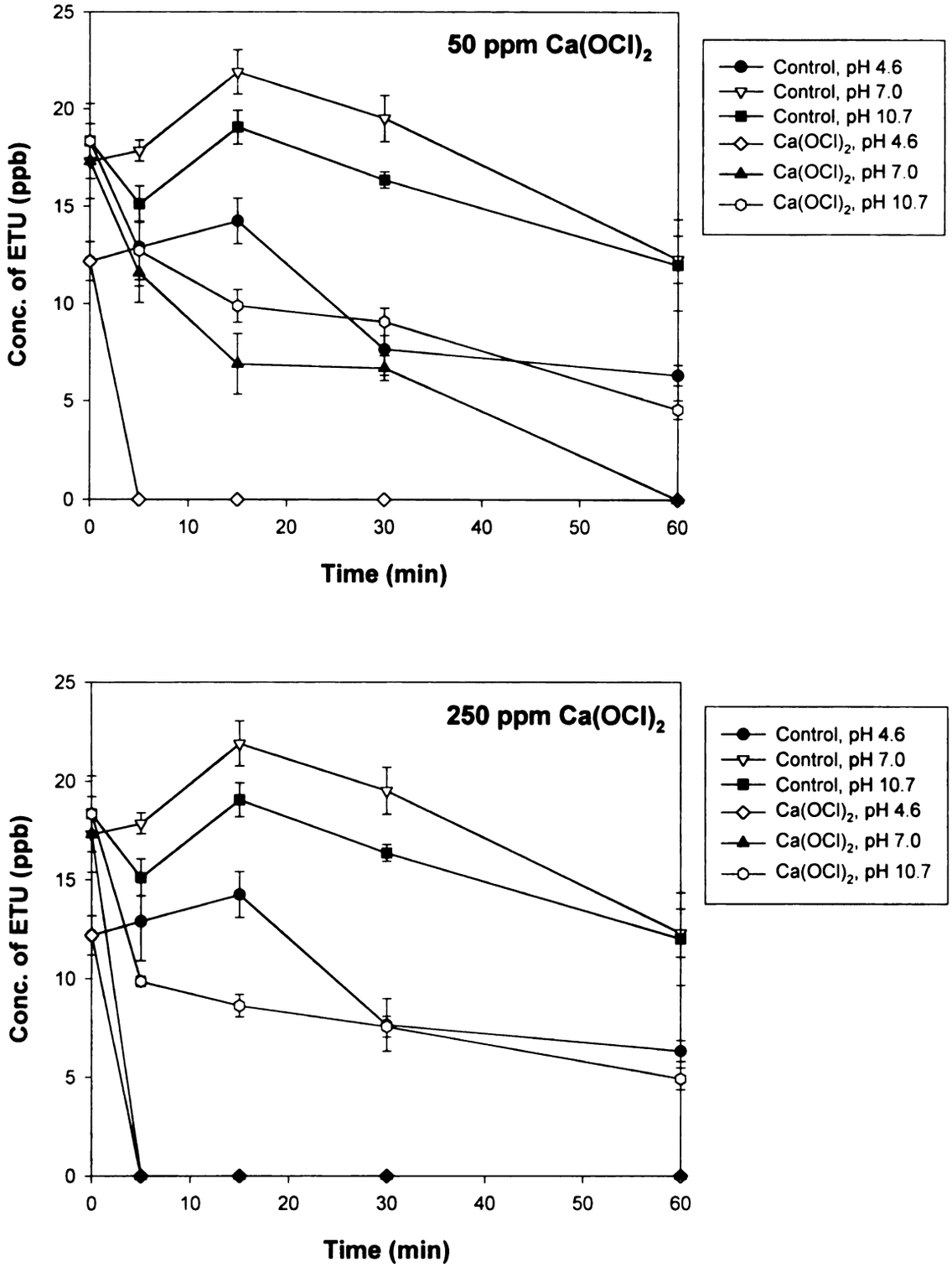


Figure 34. Effect of $Ca(OCl)_2$ on the concentration of ETU with time.

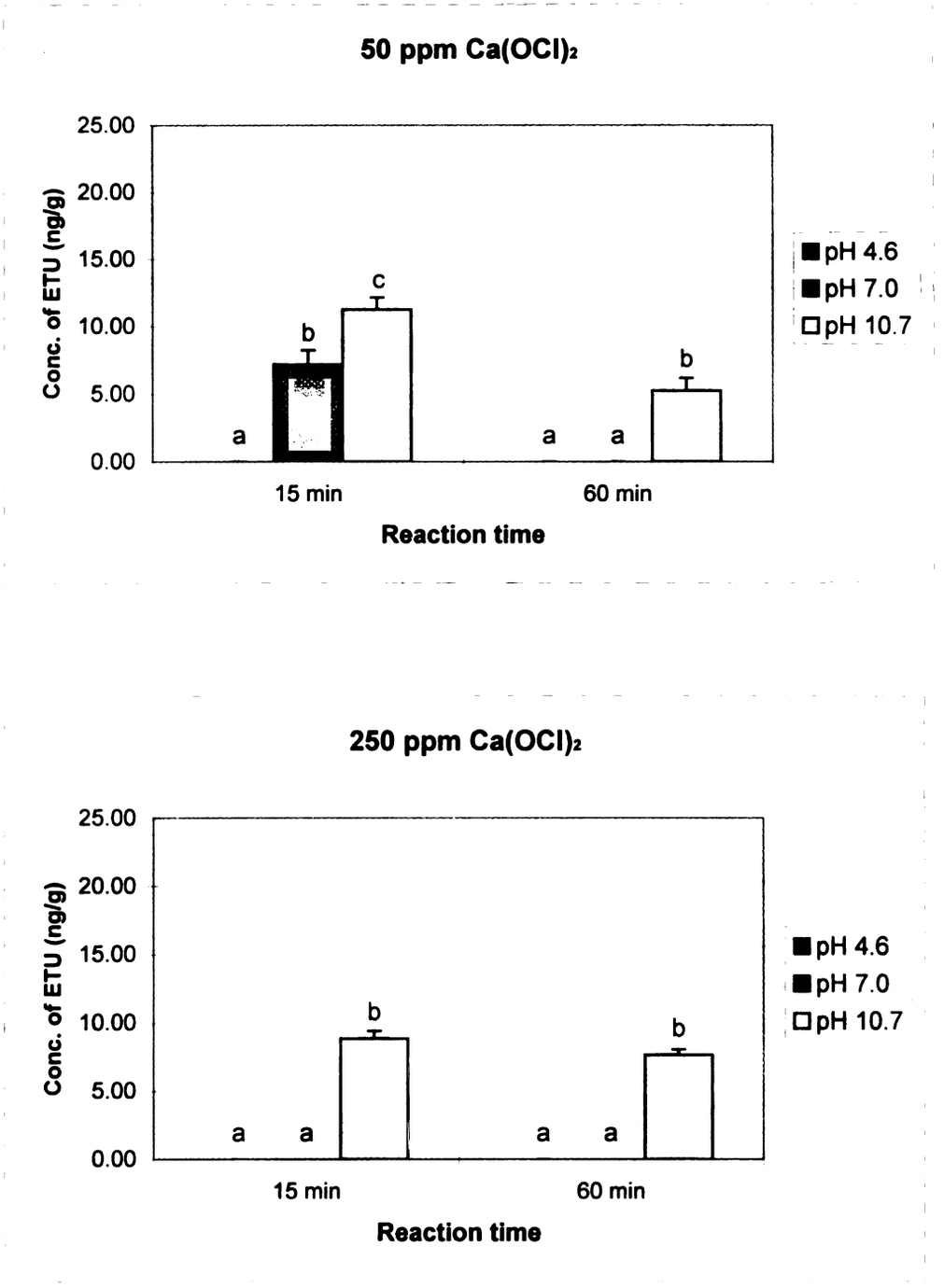


Figure 35. Effects of pH and reaction time on the conversion of Mancozeb into ETU in $Ca(OCl)_2$ treatments.

* Values with same letters are not significantly different (p <0.05).

significantly ($p<0.05$) increased the rate of degradation of ETU. No ETU was detected in either 50 or 250 ppm calcium hypochlorite treatment at pH 4.6 and 7.0 after 60 minutes (Figure 35). However, in 250 ppm chlorine solution, 51% of ETU residue still remained after 60 minutes. Again, the most effective pH for the degradation of ETU was chlorination in pH 4.6 solution, while pH 10.7 was the least effective treatment.

(III) Degradation of ETU by Chlorine Dioxide

Degradation of ETU by chlorine dioxide showed a pattern similar to calcium hypochlorite treatment (Figures 36–37). As can be seen in Figure 36 and 37, when chlorine dioxide and chlorine were used to degrade ETU residues, the required amount of chlorine dioxide was lower than that of chlorine. Maximum degradation of ETU by chlorine dioxide was observed at pH 4.6. No ETU residues were detected at either 5 or 10 ppm chlorine dioxide at pH 4.6 after 5 minutes (Figure 36). The effects of pH and reaction time on the degradation of ETU in solution are illustrated in Figure 37. Chlorine dioxide at 10 ppm significantly ($p<0.05$) increased the rate of degradation of ETU in all pH ranges. However, all ETU residues were completely degraded at 10 ppm chlorine dioxide in three pHs after 60 minutes so there was no significant ($p<0.05$) difference at this point. The most effective pH on the degradation of ETU was

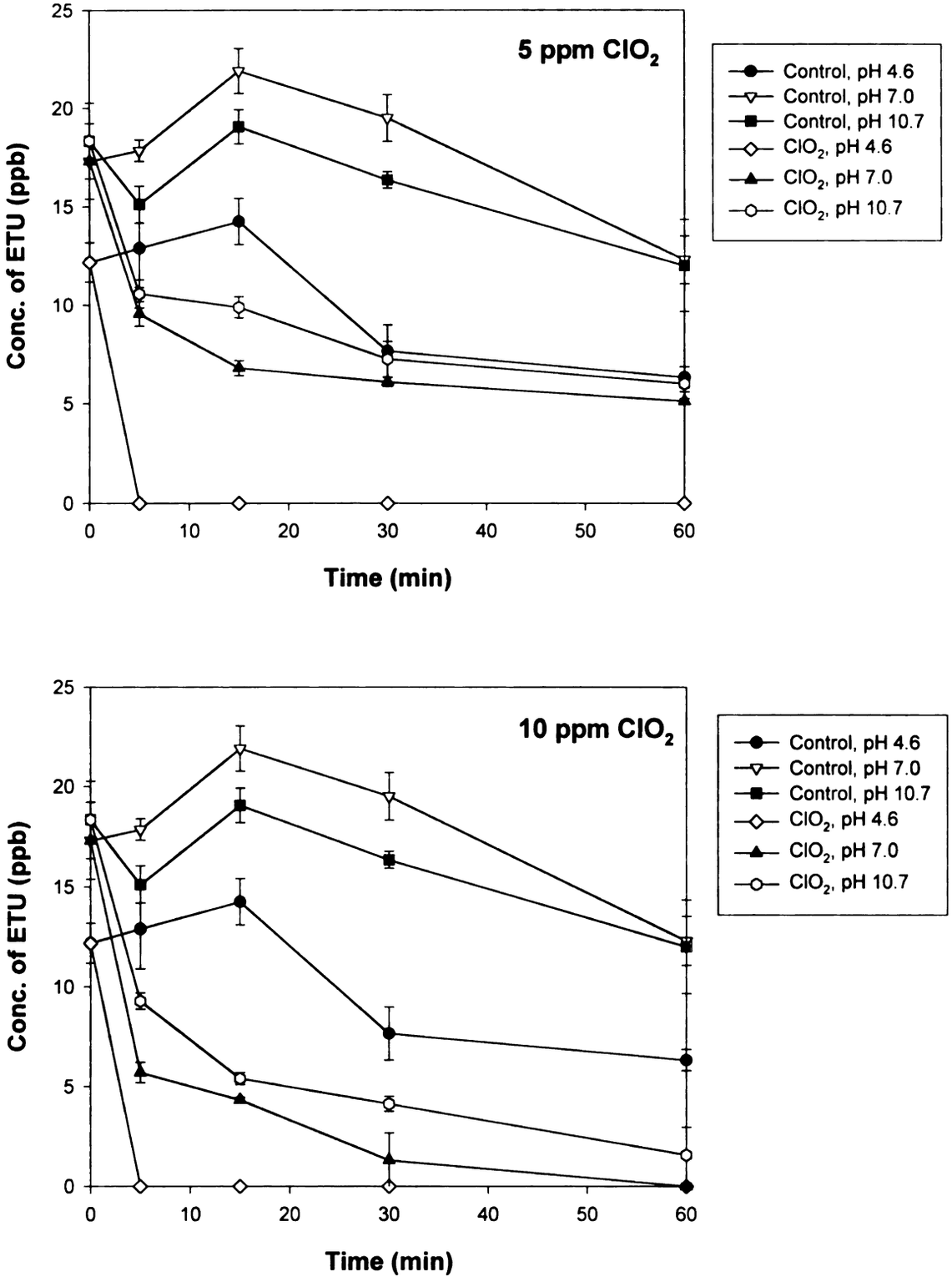


Figure 36. Effect of ClO_2 on the concentration of ETU with time.

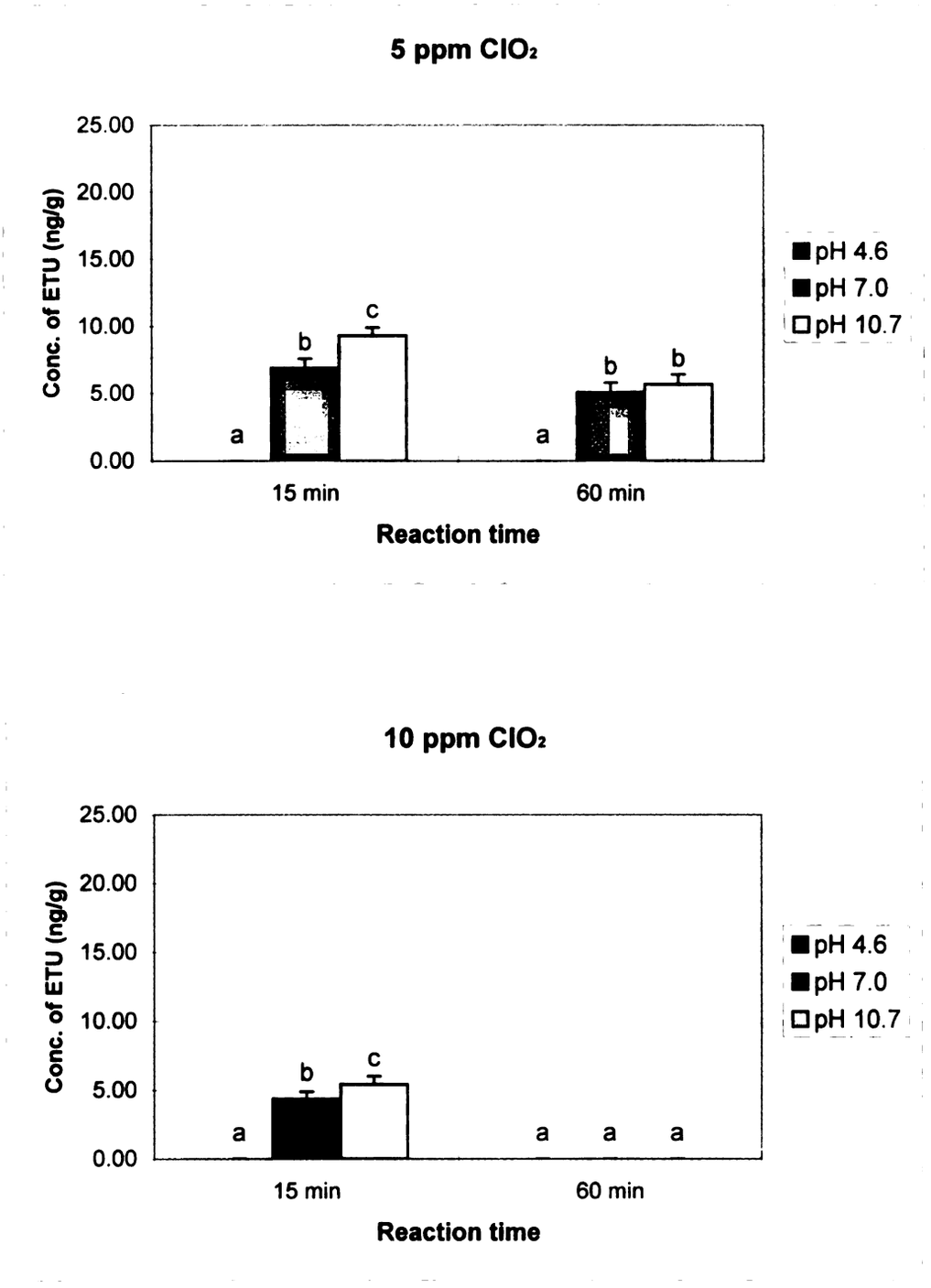


Figure 37. Effects of pH and reaction time on the conversion of Mancozeb into ETU in ClO_2 treatments.

* Values with same letters are not significantly different (p <0.05).

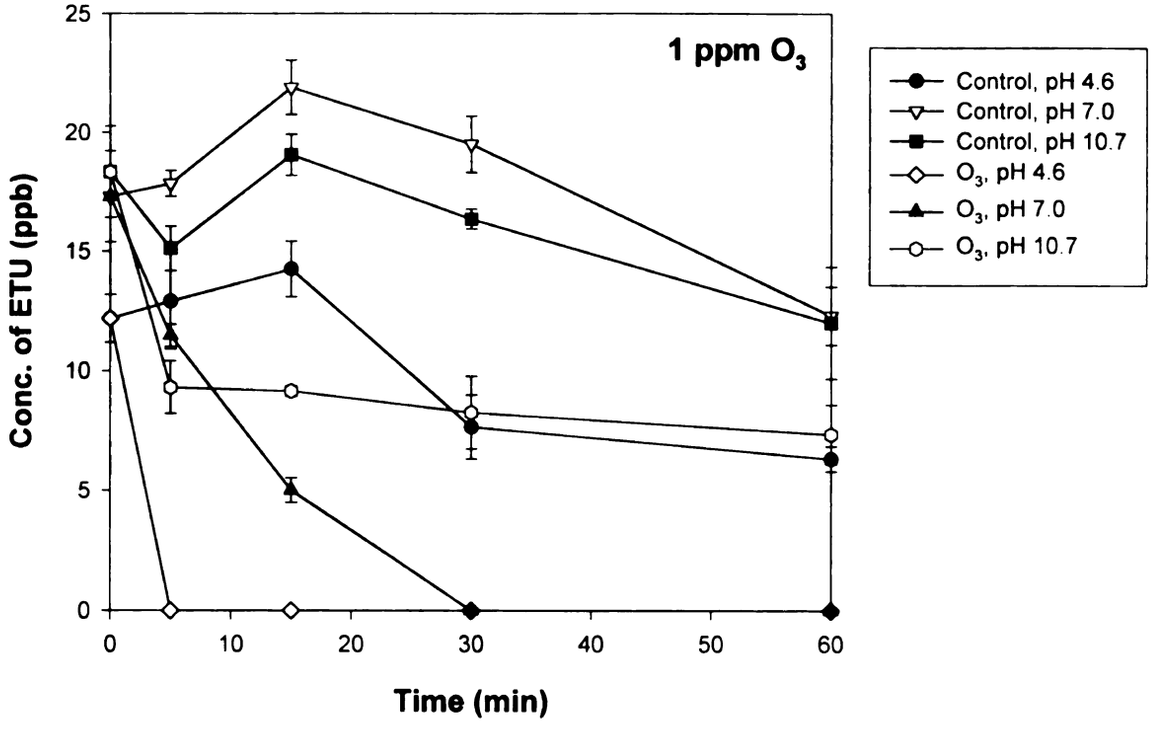
chlorine dioxide in pH 4.6 solution, while pH 10.7 was the least effective treatment.

(IV) Degradation of ETU by Ozone

Degradation of ETU by ozone was greatest at pH 4.6 and 7.0. (Figures 38–39). The ozone treatment at pH 10.7 was the least effective with degradation of 62 and 49% after 5 and 60 minutes, respectively at 1 ppm concentration (Figure 38). At 1 ppm ozone treatments, no ETU was detected after 30 minutes at either pH 4.6 or 7.0. Ozonation at 3 ppm significantly ($p<0.05$) increased the rate of degradation of ETU in all three pHs. Ozone showed the most powerful effects on the degradation of ETU compared to the other agents, with complete degradation of all of ETU within the first 15 minutes (Figure 39).

(V) Degradation of ETU by Hydrogen Peroxyacetic Acid

Maximum degradation of ETU by HPAA was observed at pH 4.6, whereas 10.7 and pH 7.0 showed the least effectiveness (Figures 40–41) 5 and 50 ppm after 15 minutes. In 5 and 50 ppm HPAA treatments, no ETU was detected at both pH 4.6 and 10.7 after only 5 minute reaction time (Figure 40). In 5 ppm HPAA treatment, between 46 and 30% of initial ETU remained after 5 and 30 minutes at pH 7.0. However, increased reaction time (60 minutes) completely degraded all ETU



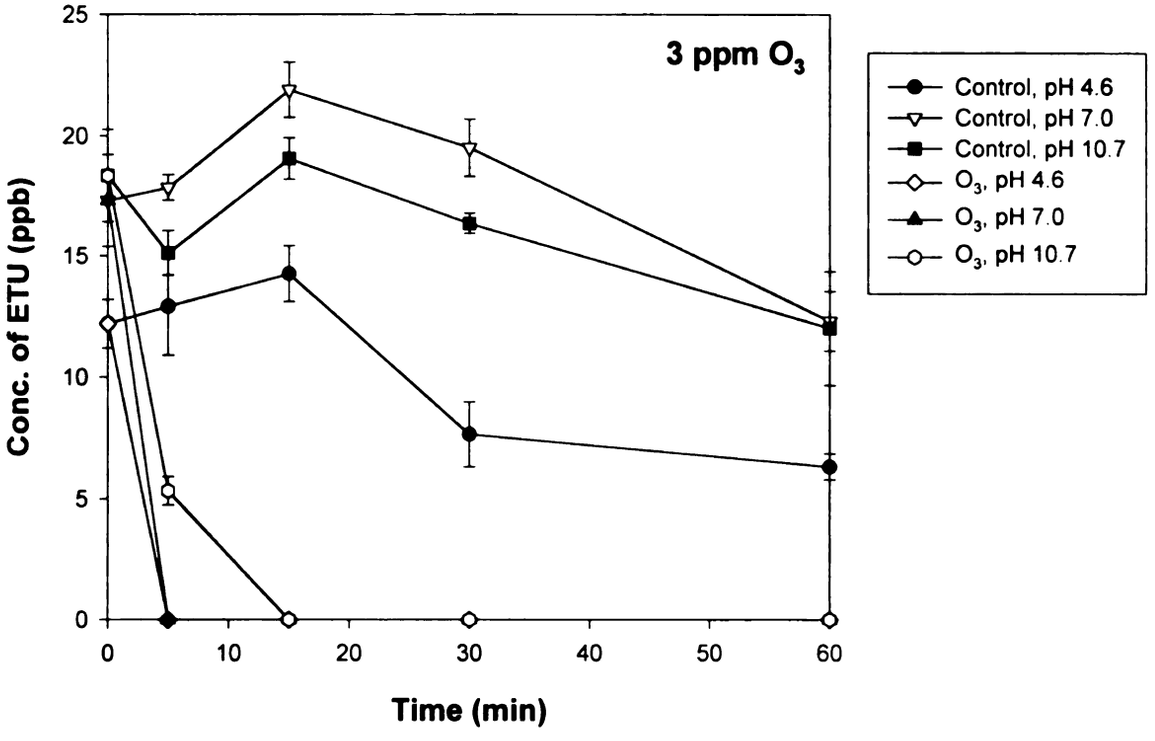


Figure 38. Effect of O_3 on the concentration of ETU with time.

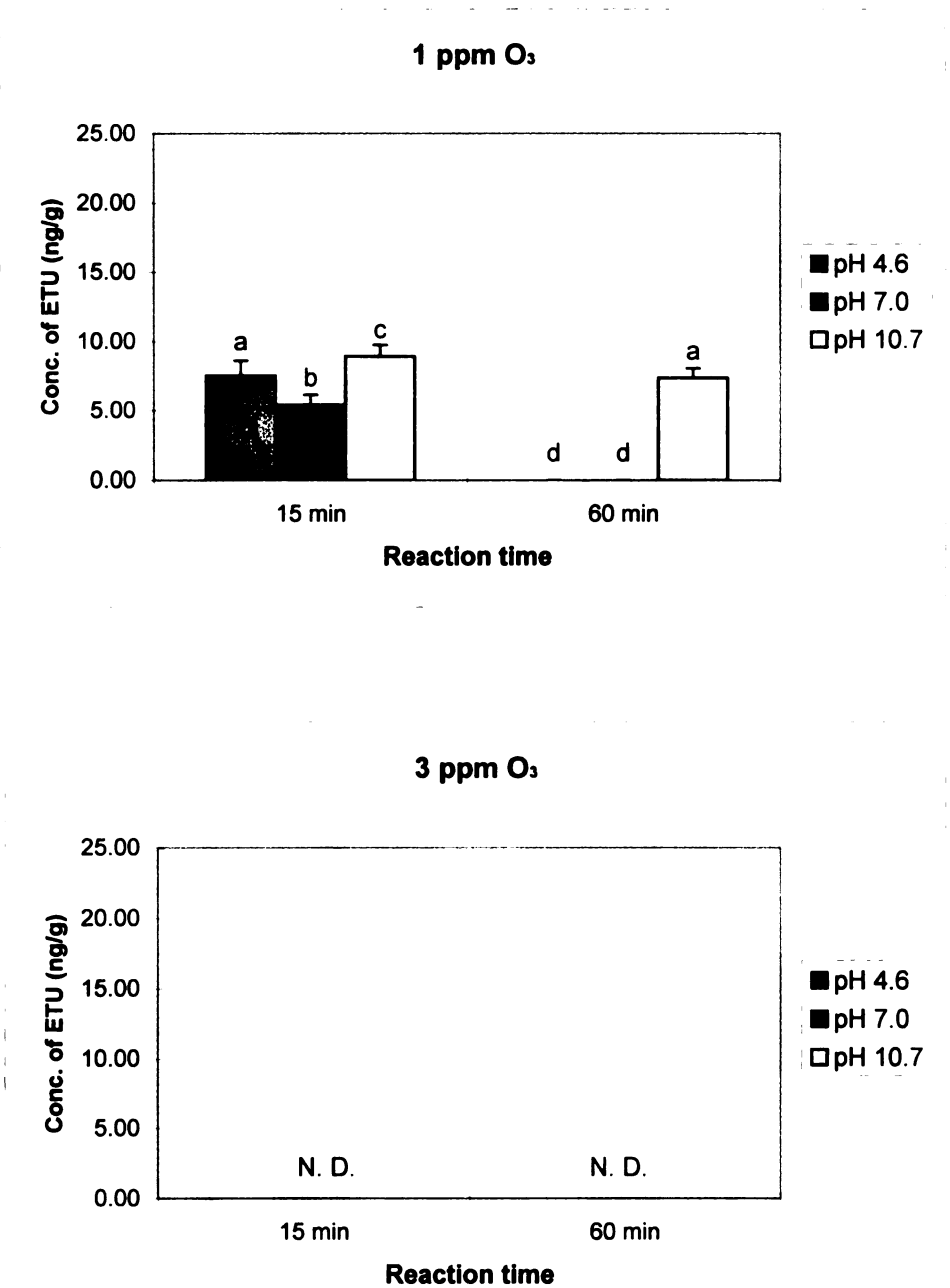


Figure 39. Effects of pH and reaction time on the conversion of Mancozeb into ETU in O_3 treatments.

* Values with same letters are not significantly different (p <0.05).

* N. D. = None detected.

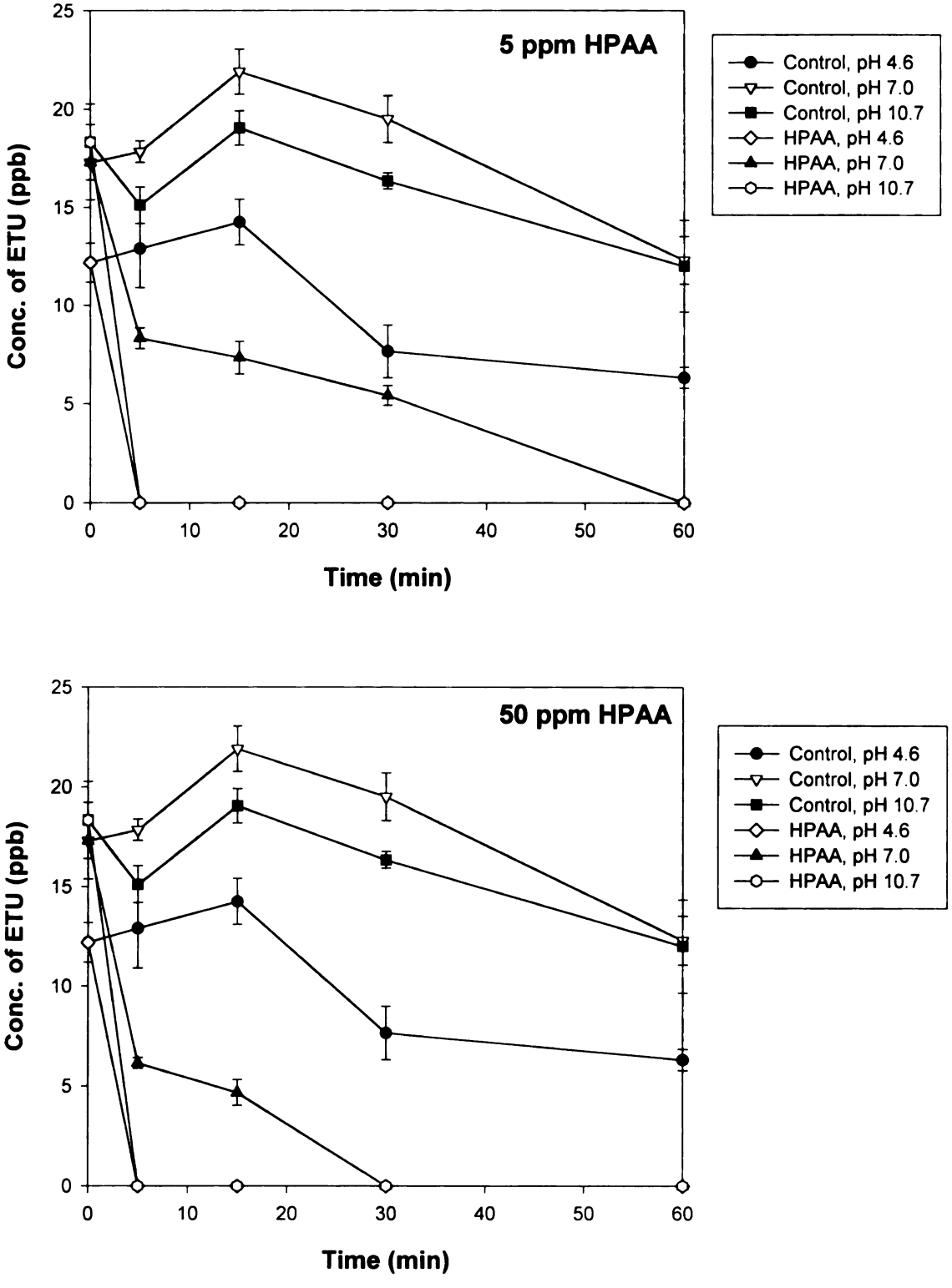


Figure 40. Effect of HPAA on the concentration of ETU with time.

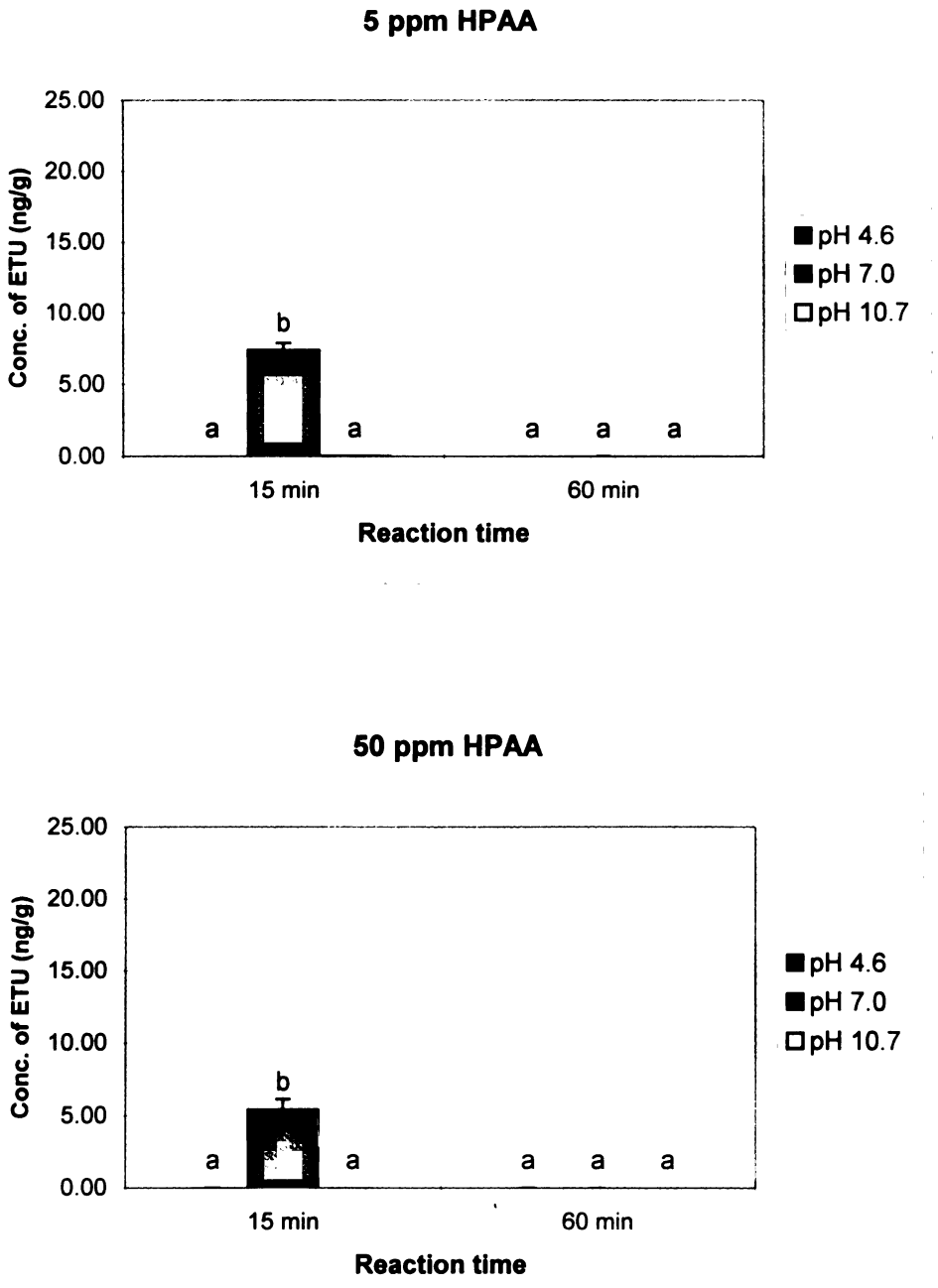


Figure 41. Effects of pH and reaction time on the conversion of Mancozeb into ETU in HPAA treatments.

* Values with same letters are not significantly different (p <0.05).

residues at both concentration of HPAA (Figure 41). This indicate that longer contact time with oxidizing agents play an important role in the reduction of pesticide residues.

The rate of degradation of the EBDC to ETU is influenced by temperature, reaction time and pH of the system (Marshall, 1977). A model system study was developed which was shown to be effective in monitoring the degradation or disappearance of mancozeb through the use of various pH, temperature, chlorine and chlorine dioxide, ozone and HPAA treatments. These treatments indicated the potential for a removal of pesticide residues on the fruit and in processed products.

SUMMARY & CONCLUSION

The objective of this study was to determine the effectiveness of chlorine and chlorine dioxide, ozone and HPAA treatment on the dissipation of mancozeb and ETU in buffered solution. A model system was developed, which was shown to be effective in monitoring the degradation or disappearance of mancozeb through the use of various pH, temperature, chlorine and chlorine dioxide treatments. Calcium hypochlorite, chlorine dioxide, ozone and HPAA treatments were effective in reducing mancozeb and ETU residues. The rate of degradation of mancozeb by chlorine and chlorine dioxide was dependent on pH, with pH 4.6 being the most effective. Mancozeb residues decreased 40–100% with chlorine and chlorine dioxide treatments. Degradation of ETU by calcium hypochlorite and chlorine dioxide was greatest at pH 4.6 and lowest at pH 10.7. Chlorination at pH 4.6, yielded no ETU residues for both calcium hypochlorite and chlorine dioxide. Chlorine dioxide gave excellent degradation effects at lower concentrations than liquid chlorine. Mancozeb residues in model system solutions decreased 56–100% with ozone treatment. At 3 ppm ozone treatment, no ETU residues were detected at all three pH ranges after 15 minute reaction time. HPAA was also effective in degrading the mancozeb residues. Degradation of ETU by

HPAA was greatest at pH 4.6 and no ETU residues remained after 5 minutes at both 5 and 50 ppm. ETU residues were quickly degraded under acidic conditions with both ozone and HPAA treatments.

The results showed that all oxidizing agents used in this study gave excellent degradation of pesticide residues depending on pH and temperature. These experiments indicated the potential for the removal of pesticide residues on fruit and in processed products.

CHAPTER II. STUDIES ON THE DEGRADATION OF PESTICIDES IN SPIKED APPLES

INTRODUCTION

Pesticide use in agriculture over the last several decades has proven to be a great benefit to the production of our food supply. Pesticide use has improved both the efficiency of growing crops and the quality of food produced. This has, in turn, lowered the cost of the household food budget. However, along with the benefits emerged the potential effect of trace amounts of pesticide residues remaining on some commodities at the time of sale to the general public. There has recently been concern by consumer groups demanding assurance from the agricultural community that the food we eat is indeed safe.

The pesticide selected in this study was mancozeb (Dithane 75 DF®), which is an ethylenebisdithiocarbamate (EBDC). EBDCs are fungicides which are frequently used for the control of fungal diseases in a wide range of fruits and vegetables. EBDCs are highly active and reliable nonsystemic fungicides and have gradually replaced the older products, establishing higher levels of disease control (Uesugi, 1998). Compared with nonsystemics, the systemic fungicides are approximately twice as valuable in terms of effects. Their success relies as much on their technical strength as on their low cost. In many cases, their use of multi-site modes of action is essential in mixtures or program

applications with more sophisticated products in order to control resistance (Uesugi, 1998). Concerns about the safety of mancozeb, for example, have been rebutted, but if they had been accepted and mancozeb withdrawn from the market, several crops would have been devastated by fungal attack and many systemic fungicides exposed to increased problems of resistance risk. However, EBDCs are subject to decomposition at elevated temperatures and high humidity and yield ethylenethiourea (ETU) as the principal metabolite in foods which contain EBDC's (Lenza–Rizos, 1990). ETU is also formed during the dissipation of the EBDC fungicides and the conversion rate or degradation of ETU is greater than its formation rate.

Chlorine, chlorine dioxide, ozone and hydrogen peroxyacetic acid (HPAA) have been employed historically for the oxidation of organic compounds at water treatment plants and were consequently investigated for their capacity to degrade organic pesticides. Chlorine and ozone treatments have shown to be effective on reduction of azinphos–methyl, captan, formetanate–hydrochloride and propargite residues in apples and apple products (Ong *et al.*, 1996; Cash *et al.*, 1997).

The previous solution laboratory studies were used to determine the optimum parameters for the degradation of mancozeb and ETU. These results were then used to determine the conditions that were subsequently employed for these laboratory whole fruit studies. The

objective of this study was to reduce or eliminate mancozeb and ETU residues in mancozeb spiked apples. The effectiveness of chlorine, chlorine dioxide, ozone and HPAA on the reduction of mancozeb and ETU residues were also examined based on previous model system studies.

MATERIALS AND METHODS

MATERIALS

A. Apple Samples

Mature Golden Delicious apples were obtained from a commercial orchard in Onondaga, Michigan. These apples had not been sprayed with mancozeb during growing seasons. The fruits were hand picked randomly from various regions of the trees, thoroughly mixed and stored at 4°C until they were prepared for residue analysis.

B. Reagents

(I) Solvents

All organic solvents used for the preparation of stock solutions, extraction, gas chromatography (GC) and high performance liquid chromatography (HPLC) were distilled-in-glass residue grade or better. Acetone and methylene chloride were obtained from J. T. Baker, Co. (Phillipsburg, NJ).

(II) Chemicals

Mancozeb standard was obtained from Rohm & Haas (Philadelphia, PA). ETU standard was obtained from Aldrich Co. (Milwaukee, WI). The stock solutions of mancozeb and ETU were prepared in distilled water at concentration of 100 μg/100 ml. The standards were protected from light and stored in refrigerator at 4°C. Chlorine solutions were prepared from calcium hypochlorite (Aldrich, Milwaukee, WI) as a source of chlorine. Sodium thiosulfate, sodium sulfate, potassium iodide, potassium indigo trisulfonate were all reagent grade.

C. Glassware

All glassware was thoroughly washed with detergent and warm water, then rinsed with distilled water. The glassware was then rinsed with acetone and placed in an oven at 400°C overnight before use.

METHODS

The model system solution studies were used to determine the optimum parameters for the degradation of mancozeb and ETU. These results were then used to determine the conditions that were

subsequently employed for these laboratory whole fruit studies. Based on model system studies, (i) calcium hypochlorite at two concentrations (50 and 500 ppm), chlorine dioxide at two concentrations (5 and 10 ppm), ozone at two concentrations (1 and 3 ppm), and hydrogen peroxyacetic acid at two concentrations (50 and 500 ppm) (ii) one ambient pH of 6.7 (distilled water) (iii) one ambient temperatures (21°C) were selected. Degradation of the mancozeb was studied over a 30 minute period because the typical water contact time in a commercial plant is about 10–15 minutes and under normal conditions would rarely exceed 30 minutes. There were three replications per treatment. Samples were taken at appropriate intervals for analysis of mancozeb and ETU residues.

Calcium hypochlorite stock solution (5000 ppm) and HPAA stock solution were used as a chlorine and peroxyacetic acid sources. Chlorine dioxide and ozone were generated in the laboratory. The detailed preparation and determination methods are given in the Method Section of Chapter I.

A. Sample Extraction

Apples were coated by carefully dipping 5 ml of water containing 1 μg/ml and 10 μg/ml of mancozeb onto each individual apple surface. The water was allowed to evaporate and then the apples (five at a time)

were placed in 500 ml of distilled water, at room temperature and desired concentration of solution of calcium hypochlorite (50 and 500 ppm), chlorine dioxide (5 and 10 ppm), ozone (1 and 3 ppm) or hydrogen peroxyacetic acid (50 and 500 ppm). At the predetermined reaction time (0, 3, 15 and 30 min) the apples were removed, the surface extracted with 20 ml of water and analyzed for mancozeb residues by GC. Dipping solutions were also analyzed by GC for mancozeb residues.

B. Pesticide Residue Analyses

(I) Mancozeb

Mancozeb residues were analyzed as carbon disulfide (CS_2) by gas liquid chromatographic headspace analysis (Ahmad *et al*, 1995). Twenty mls of sample were transferred at 0, 5, 15, and 30 minutes interval into sample bottles. A 0.5% 0.1 M sodium thiosulfate solution was added to the samples at the appropriate time to quench the reaction. Forty mls of 1.5% stannous chloride in 5 M HCl were added and immediately sealed with a crimped septum. Fifty µls of a 1 mg/ml thiophene solution were injected into each bottle and incubated at 70–80°C in a water bath for 15 minutes. Bottles were removed and agitated for 2 minutes by hand. Bottles were replaced in the water bath with repeated shaking for 1 hour. A 100 µl sample was removed with a gas tight syringe from the bottle headspace, and injected into the GC.

(II) ETU

ETU residues were determined using a modification of the HPLC method published by Ahmad *et al.* (1995). Twenty mls of sample were weighed into an Erlenmeyer flask, then 8 g of potassium fluoride and 0.6 g of ammonium chloride were added. This mixture was extracted with 50 ml methylene chloride 2 times. The methylene chloride layer was passed through a bed of 25 g anhydrous sodium sulfate collected in a Zymark Turbovap tube and evaporated to dryness on an automated Zymark Turbovap evaporator (Zymark Inc., Hopkin, MA) at 40°C. The residue was dissolved in 3 ml distilled water and 50 µls were injected into an HPLC column.

C. Chromatographic Analyses

(I) Mancozeb

Mancozeb residues were detected and quantified using a Hewlett Packard Series II 5890 gas chromatograph (GC) equipped with a flame photometric detector (FPD) in the sulfur mode. The GC was equipped with a Supel-Q-Plot fused silica capillary column (30 m long x 0.53 mm ID) with a film thickness of 0.25 µm (Supelco Inc., Bellefonte, PA). The oven temperature was 80°C, while the injector and detector temperatures were 230°C and 300°C, respectively. Helium and nitrogen

were used as the GC carrier gas and makeup gas, respectively. Carrier gas flow through the column was 20 ml/min. Integration was carried out with HP Chemstation software interfaced to the GC.

(II) ETU

ETU residues were detected and quantified using a Waters liquid chromatograph with a Hypersil BDS C_{18} column (250 mm x 4.6 mm, 5 μm particles), a Hypersil BDS C_{18} guard column (10 mm x 4.6 mm, 5 μm particles) and UV detector set at 240 nm. The mobile phase was 0.72% butylamine in distilled water at pH 3.0–3.2. A M–45 Waters HPLC pump (Waters Associates, Inc., Milford, MA.) was used for solvent delivery at a flow rate of 0.5 ml/minutes. After the system was stabilized (about 1 hour from initial warm–up), 75 μl samples were injected via a Rheodyne syringe loop injector (50 μl loop) for analysis. Integration was carried out using 3390 A Hewlett Packard integrator.

D. Calculation of Pesticide Residue Concentration

Mancozeb and ETU residue concentrations in solution were calculated based on the area of the integrated peaks of the samples compared with known concentrations of analytical standard of the respective pesticides. Standard curves of the mancozeb and ETU were

plotted and least square linear regression was obtained using a Microsoft Excel (Microsoft Corporation, Redmond, WA) software.

The residue concentrations were calculated based on the following formula:

(a) Mancozeb residue in μg/ml

$$\text{ppm} = \frac{\text{ng Mancozeb}}{\text{mg sample injected}}$$

where, ng Mancozeb was derived from standard curve

$$\text{mg sample injected} = \frac{20\text{g}}{\text{headspace volume sample – containing reaction vial} \times \mu\text{L injected}}$$

where, headspace volume of sample – containing reaction vial = 40 mL

(b) ETU residue in μg/ml

$$\frac{\text{Conc. of ETU in sample extract based on std. Curve}(\mu\text{g/g}) \times \text{Vol. final extract (3 ml)}}{\text{Weight of sample analyzed (20 g)}}$$

E. Statistical Analyses

All determinations were replicated three times. Mean standard deviations, mean square errors, two factor ANOVA, correlation and interaction of main effects were calculated using Sigmastat computer

software 1.0 (Jandel Corp., San Rafael, CA). Appropriate comparisons were made using Student–Newman–Keuls Method for multiple comparisons. A $p<0.05$ was considered statistically significant.

RESULTS & DISCUSSION

A. Recovery Study

Based on model system studies, whole fruit studies were conducted. To determine the extraction efficiency of the mancozeb on apples by presented extraction techniques, five apples (about 700 g) were fortified with mancozeb at two concentration levels (1 and 10 μg/ml). Table 5 gives the percent recoveries obtained from fortified apples. On the basis of the regression equation, average recoveries of mancozeb were 84.0% at 1 μg/ml and 91.3% at 10 μg/ml spiked level.

Table 5. Recovery (%) ± SD (n = 3) for the Mancozeb on apple samples

	Recovery %		
	0.01 μg/ml spiked	1 μg/ml spiked	10 μg/ml spiked
# 1	88.3	87.7	89.7
# 2	79.2	83.8	94.1
# 3	76.9	80.6	90.2
Mean	77.3 ± 1.7	84.0 ± 3.6	91.3 ± 2.4

The method of detection limit (MDL) for mancozeb was determined to be 0.01 μg/ml. The percent recoveries at MDL are presented in Table 5. Relatively high recoveries were obtained for all

three spiked levels. Recoveries appeared to decline when spiked at a lower level. The lower recoveries may be a result of matrix effects on extraction efficiency. Samples which contain low levels initially, are more likely to show these discrepancies (Siler, 1998).

B. Degradation of Mancozeb in Spiked Apples

Based on model system study, ambient temperature (21°C) and pH were used in this study. This experiment utilized five apples (about 700 g of apples) coated with 1 or 10 ppm mancozeb. The whole fruit spiked with mancozeb gave results similar to those found in the model system studies. Appendix 5 shows raw data for mancozeb residues in spiked apples at various time and treatments. Control studies conducted with mancozeb coated apples under the exact conditions with the treated samples but exposed only to distilled water with no other treatments showed only slight dissipation of mancozeb residues (Figure 42). This indicates that mancozeb was relatively stable in distilled water, at least, during 30 minute period. Figure 42 shows the rates of decline for mancozeb on apples. At zero reaction time, spiked mancozeb concentration was approximately 1 ppm. This decreased gradually to about 0.11 ppm and 0.01 ppm at 50 and 500 ppm calcium hypochlorite, respectively after 30 minute reaction time. In 50 ppm calcium hypochlorite treatment, almost 94% and 75% of the initial amount of

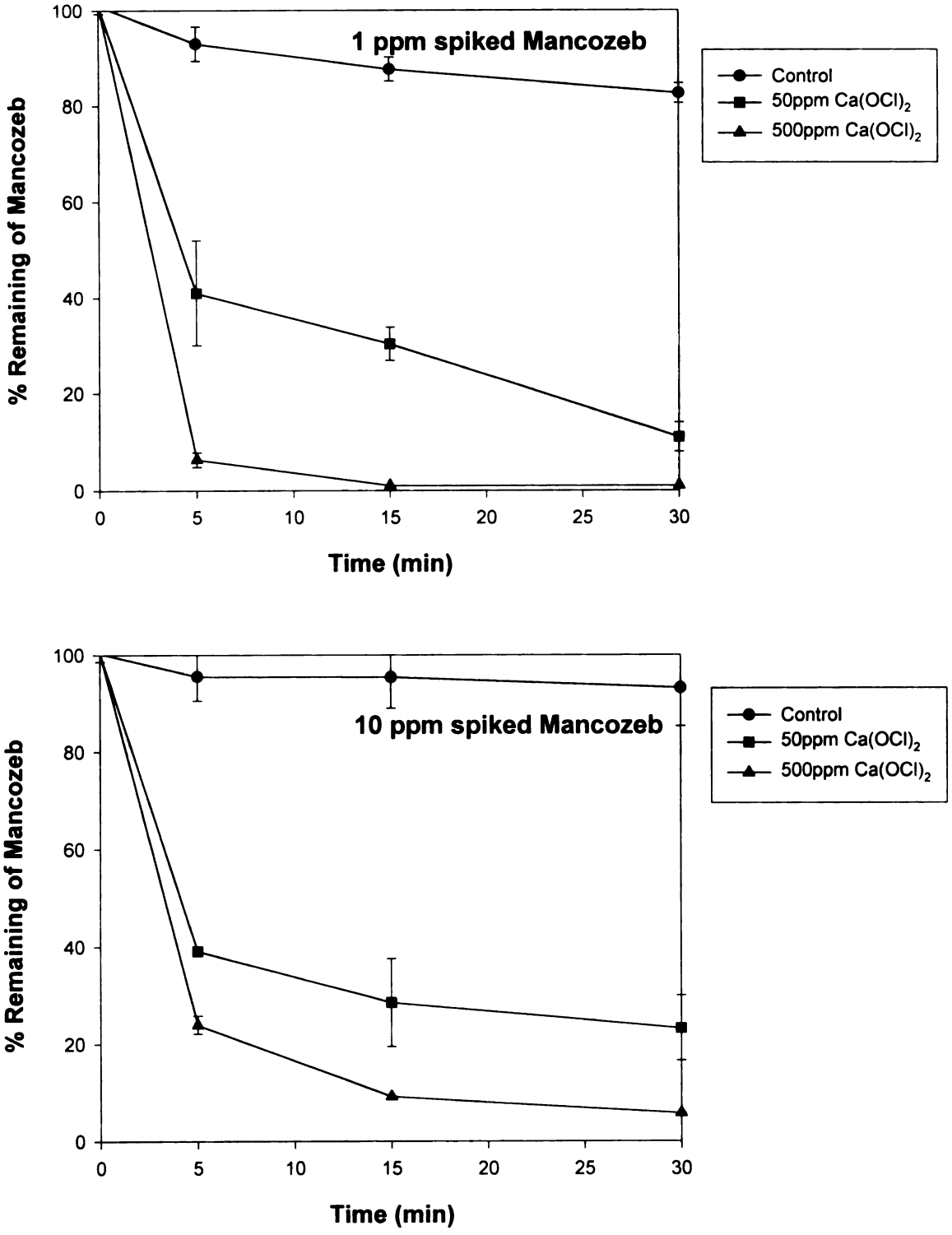


Figure 42. Effect of $Ca(OCl)_2$ on the degradation of Mancozeb in spiked apples.

mancozeb was degraded after 30 minutes at 1 and 10 ppm spiked levels, respectively. Chlorine at 500 ppm significantly ($p<0.05$) increased the rate of degradation of mancozeb. Only about 0.01 % and 0.04% of mancozeb remained at 1 and 10 ppm spiked levels after 30 minute reaction time

Degradation of mancozeb residues by chlorine dioxide is shown in Figure 43. At 1 ppm mancozeb spiked level, there was no significant difference between 5 and 10 ppm chlorine dioxide treatment and the effects were lower than calcium hypochlorite. In this case, between 34 and 32% of mancozeb remained after 5 minutes at both 5 and 10 ppm chlorine dioxide treatment, respectively. After 15 minutes, degradation of mancozeb increased up to 24 and 22%; however, there was no significant difference with reaction time. In 10 ppm mancozeb spiked level, 64 and 16% of mancozeb remained after 5 minutes and 41 and 13 % of mancozeb residues after 30 minutes at 5 and 10 ppm chlorine dioxide treatment (Figure 43). It is anticipated that residue levels would be reduced considerably by the chlorine dioxide treatment if the concentration of chlorine dioxide is increased above the 10 ppm that was used in this study.

Ozonation at 1 ppm and 3 ppm significantly ($p<0.05$) increased the rate of degradation of mancozeb in 10 ppm mancozeb spiked level (Figure 44). At 3 ppm ozone concentration, 3% of the mancozeb residue

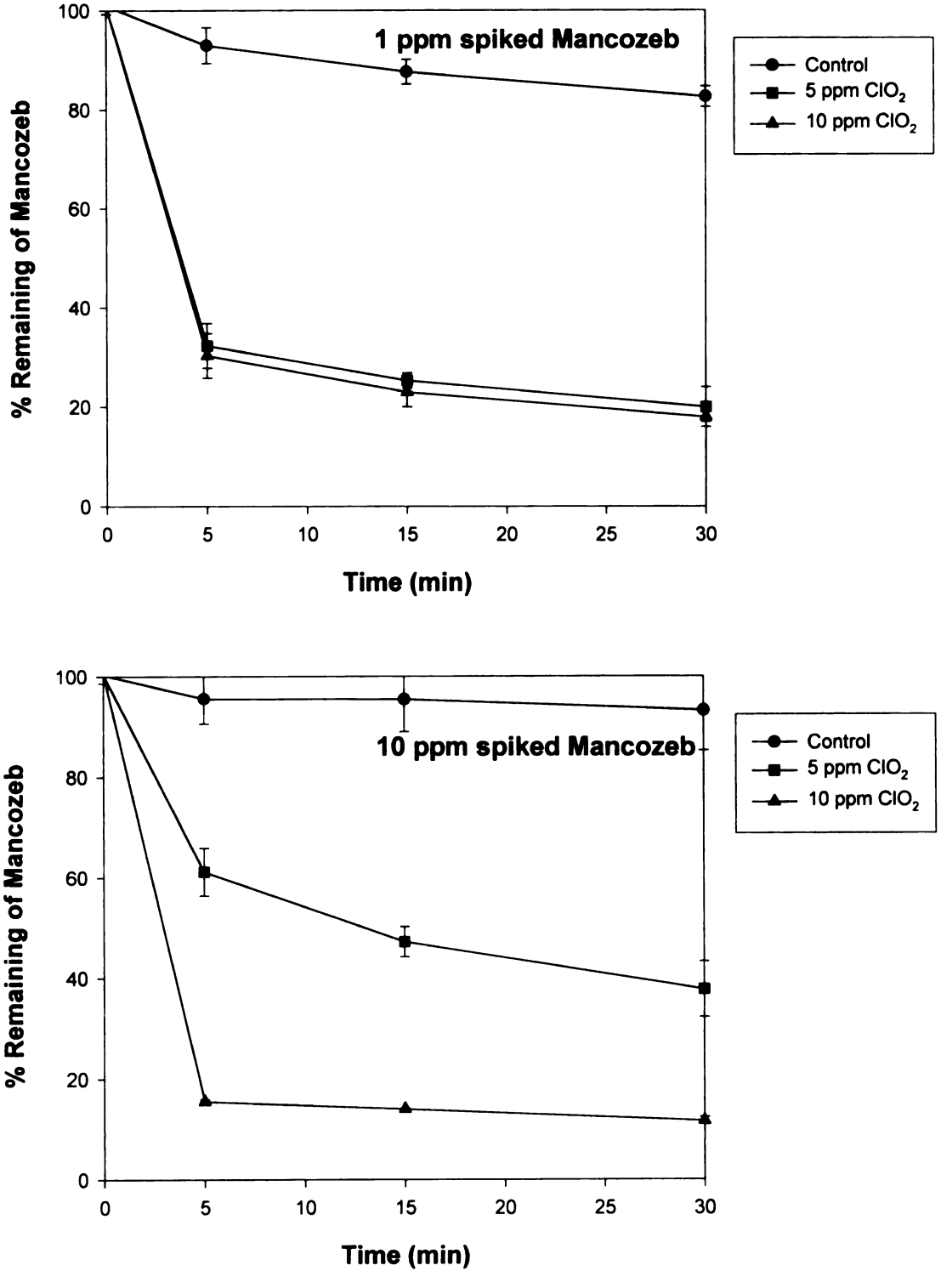


Figure 43. Effect of ClO_2 on the degradation of Mancozeb in spiked apples.

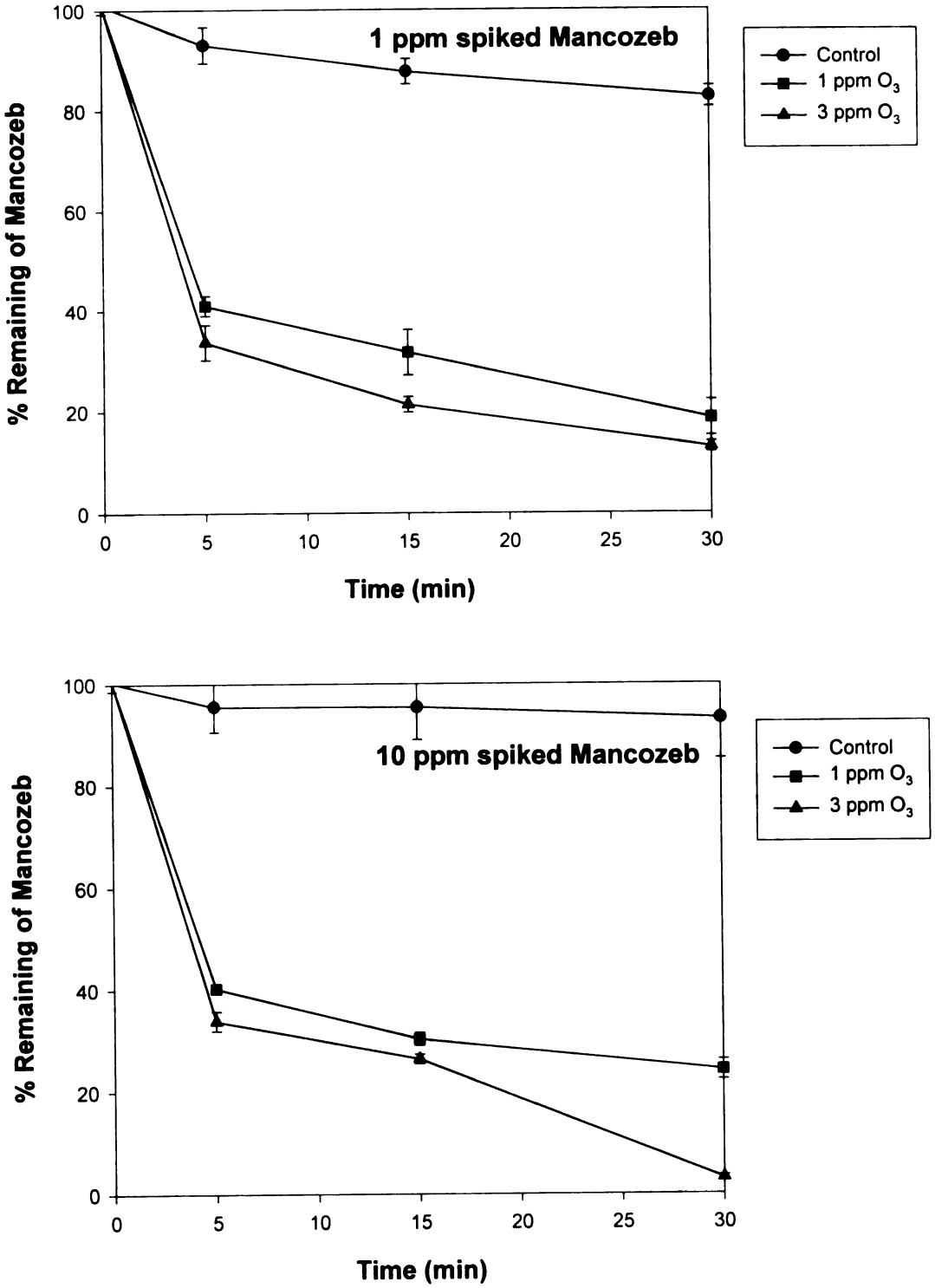


Figure 44. Effect of O_3 on the degradation of Mancozeb in spiked apples.

remained after 30 minutes at the 10 ppm spiked level, with 16% of the mancozeb residue remained at the 1 ppm spiked level. Ozone has shown to be relatively stable at neutral pH range which is close to the pH of distilled water, so this can be easily applied in commercial plants.

Degradation of mancozeb by HPAA was significantly increased at higher HPAA concentration. In 50 ppm HPAA treatment, almost 83 and 66% of the initial amount of mancozeb was degraded after 30 minutes. HPAA treatments at 500 ppm showed greater effects than 50 ppm HPAA at 1 and 10 ppm mancozeb spiked level after 30 minutes, with 99% and 98% degradation of mancozeb, respectively (Figure 45).

C. Comparison of the Effects of Various Oxidizing Agents on the Degradation of Mancozeb Residues

The effects of various oxidizing agents on the degradation of mancozeb are shown in Figures 46–47 and Tables 6–7. Mancozeb residues in all the samples were significantly reduced compared to control by exposure to various oxidizing agents. In 1 ppm mancozeb, there were no significant differences among various treatments except chlorine at 500 ppm at both 3 minute and 30 minute reaction time (Figure 46). At the 10 ppm mancozeb spiked level, 10 ppm chlorine dioxide treatment showed the best effect at 3 minute reaction time (Figure 47). With longer reaction times of 30 minutes, chlorine at 500

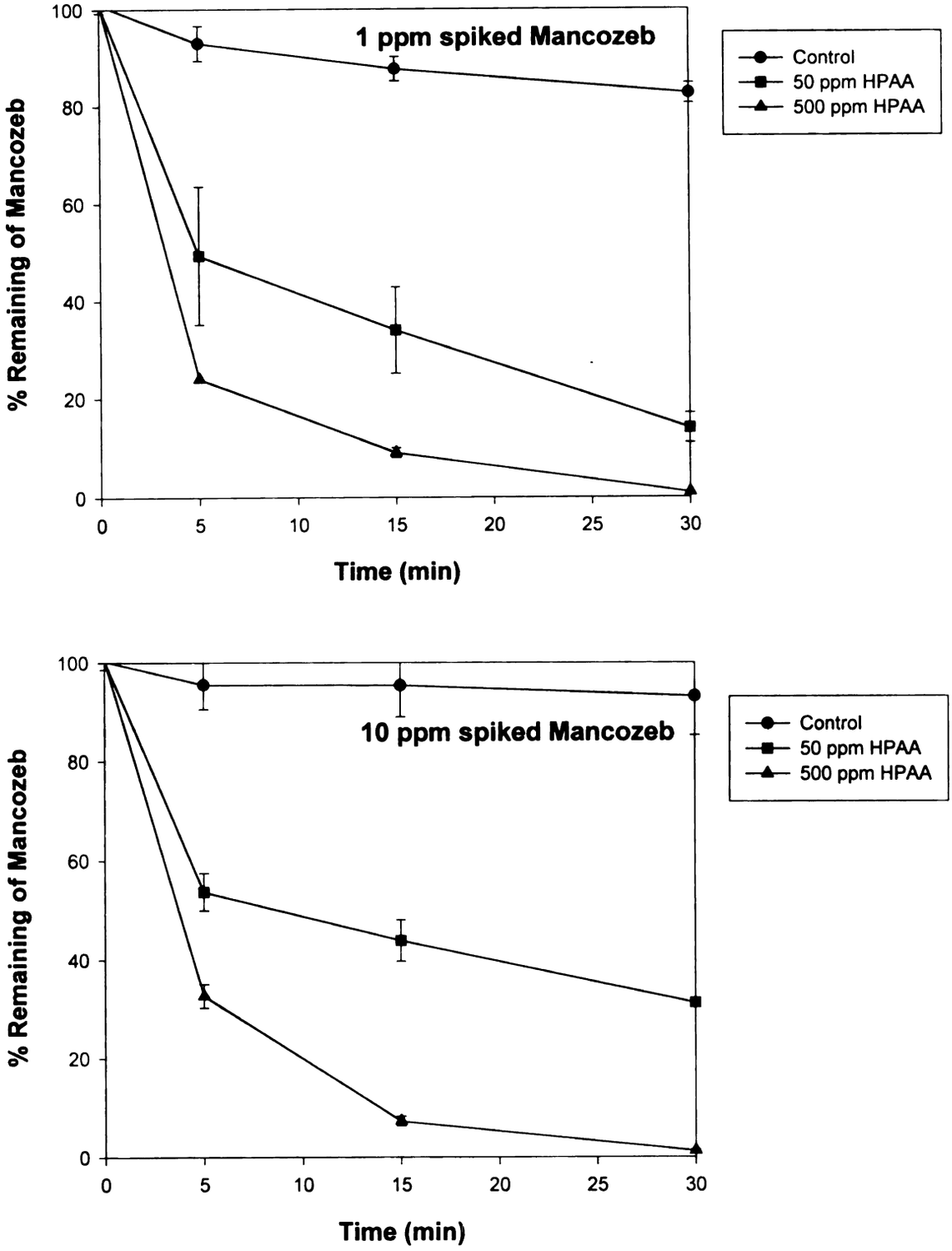
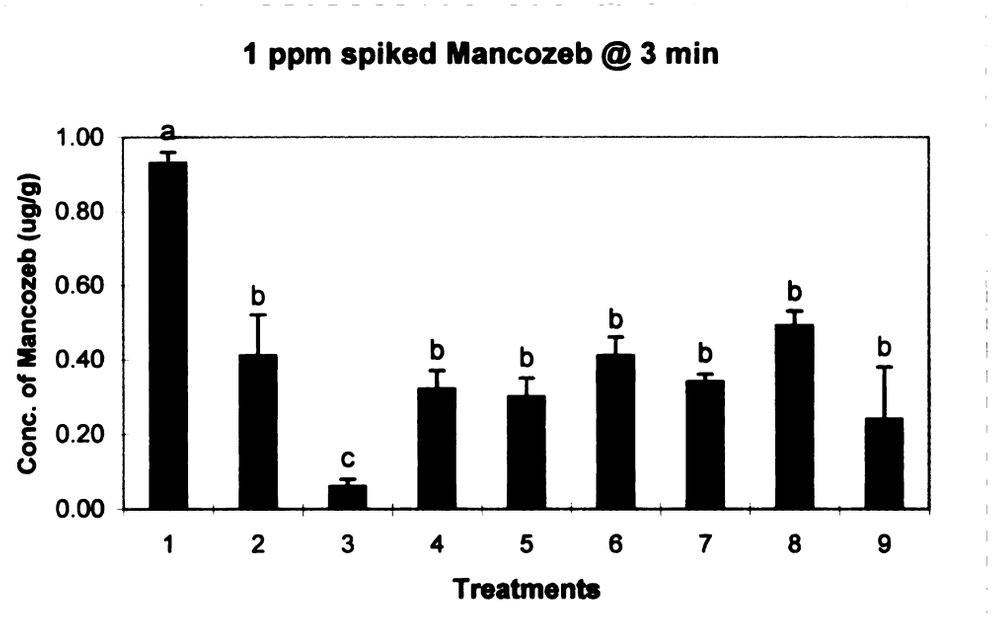


Figure 45. Effect of HPAA on the degradation of Mancozeb in spiked apples.



1. Control
2. 50 ppm chlorine
3. 500 ppm chlorine
4. 5 ppm chlorine dioxide
5. 10 ppm chlorine dioxide
6. 1 ppm ozone
7. 3 ppm ozone
8. 50 ppm HPAA
9. 500 ppm HPAA

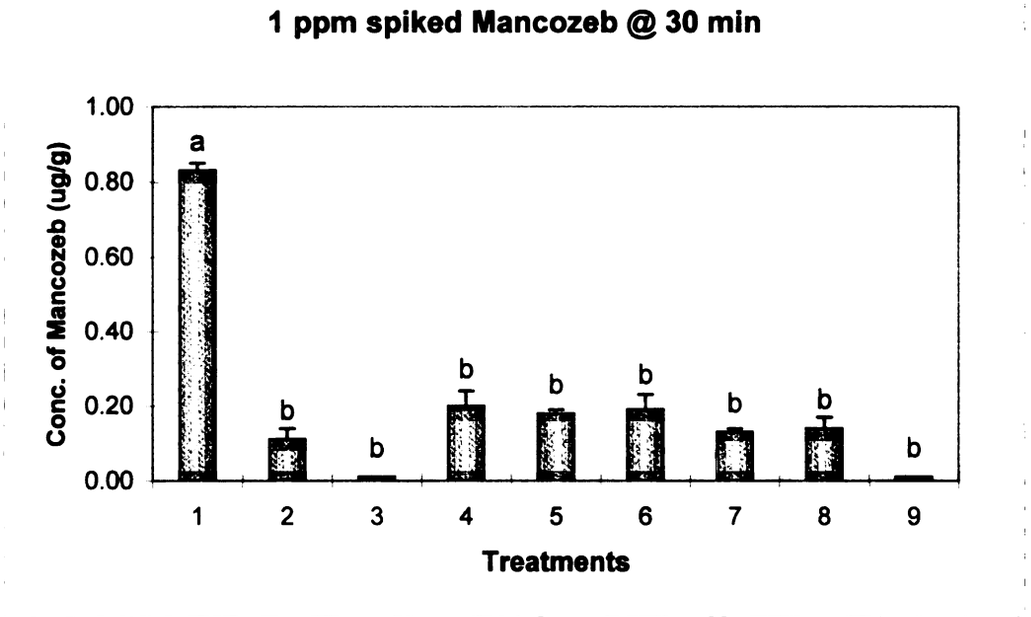
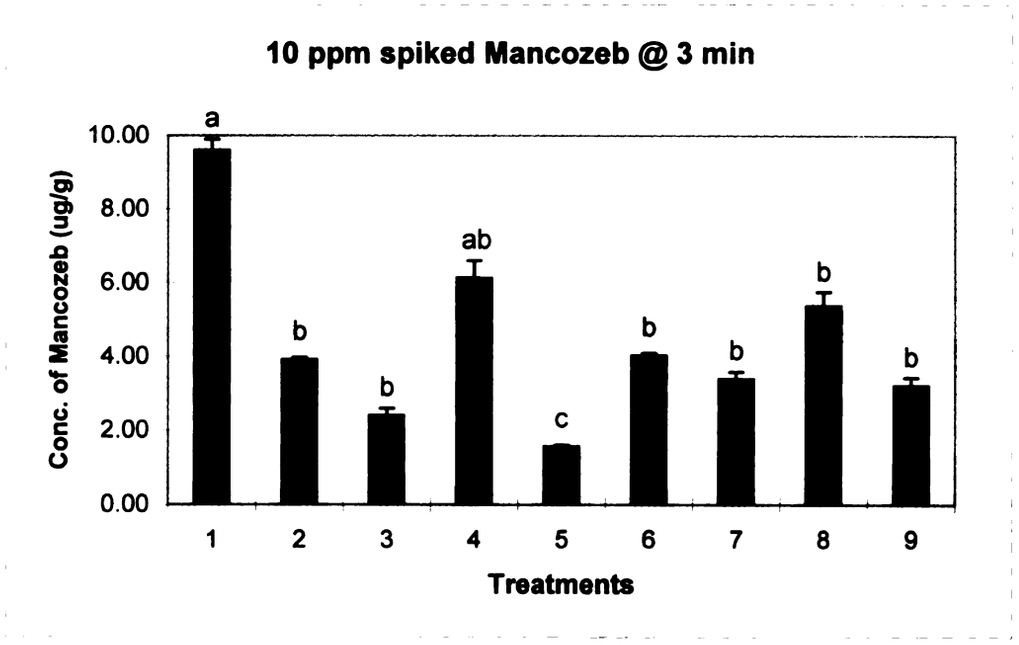


Figure 46. Comparison of various oxidizing agents on the degradation of 1 ppm Mancozeb.



1. Control
2. 50 ppm chlorine
3. 500 ppm chlorine
4. 5 ppm chlorine dioxide
5. 10 ppm chlorine dioxide
6. 1 ppm ozone
7. 3 ppm ozone
8. 50 ppm HPAA
9. 500 ppm HPAA

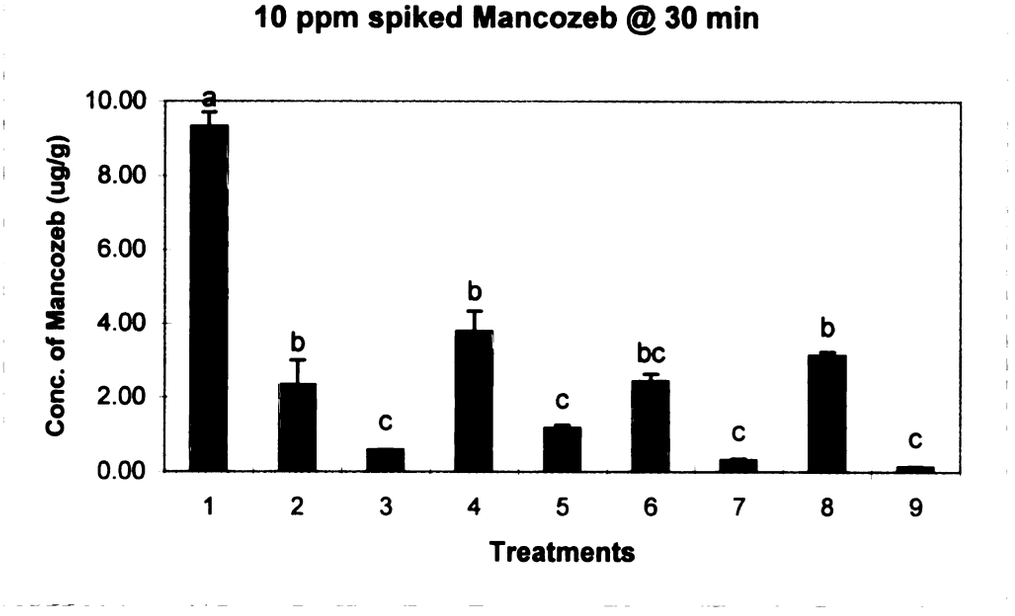


Figure 47. Comparison of various oxidizing agents on the degradation of 10 ppm Mancozeb.

Table 6. Effects of Various Oxidants on the Mancozeb Residue Concentrations and % Remaining of Mancozeb at 1 ppm Mancozeb Spiked Level

	Mancozeb Residue Conc. (ppm)		% Remaining	
	3 min	**30 min**	**3 min**	**30 min**
Control	0.93 ± 0.03[a]	0.83±0.02[a]	100	100
50 ppm $Ca(OCl)_2$	0.41 ± 0.11[b]	0.11 ±0.03[b]	44.09	13.25
500 ppm $Ca(OCl)_2$	0.06 ± 0.02[c]	0.01 ±0.00[b]	6.45	1.20
5 ppm ClO_2	0.32 ± 0.05[b]	0.20± 0.04[b]	34.40	24.10
10 ppm ClO_2	0.30 ± 0.05[b]	0.18± 0.01[b]	32.26	21.69
1 ppm O_3	0.41 ±0.02[c]	0.19 ± 0.04[b]	44.09	22.89
3 ppm O_3	0.34 ± 0.04[c]	0.13 ± 0.01[b]	36.56	15.66
5 ppm HPAA	0.49 ± 0.14[b]	0.14± 0.03[b]	52.69	16.87
50 ppm HPAA	0.24 ± 0.00[b]	0.01± 0.00[b]	25.81	1.20

Note: 1. Values are the means of triplicate determinations.

2. Values with same letters are not significantly different ($p<0.05$).

Table 7. Effects of Various Oxidants on the Mancozeb Residue Concentrations and % Remaining of Mancozeb at 10 ppm Mancozeb Spiked Level

	Mancozeb Residue Conc. (ppm)		% Remaining	
	3 min	**30 min**	**3 min**	**30 min**
Control	9.60 ± 0.59[a]	9.32 ± 0.79[a]	100	100
50 ppm $Ca(OCl)_2$	3.91 ± 0.06[a]	2.33 ± 0.67[b]	40.73	25.00
500 ppm $Ca(OCl)_2$	2.40 ± 0.19[b]	0.58 ± 0.01[c]	25.00	6.22
5 ppm ClO_2	6.12 ± 0.48[a]	3.78 ± 0.55[b]	63.75	40.56
10 ppm ClO_2	1.56 ± 0.04 [b]	1.17 ± 0.08[c]	16.25	12.55
1 ppm O_3	4.03 ± 0.06[b]	2.43 ± 0.20[b]	41.98	26.07
3 ppm O_3	3.39 ± 0.19[c]	0.31 ± 0.05[c]	35.31	3.33
5 ppm HPAA	5.37 ± 0.38[a]	3.13 ± 0.10[b]	55.94	33.58
50 ppm HPAA	3.21 ± 0.22[b]	0.13 ± 0.02[c]	33.44	1.39

Note: 1. Values are the means of triplicate determinations.

2. Values with same letters are not significantly different ($p<0.05$).

ppm, chlorine dioxide at 10 ppm, ozone at 3 ppm and HPAA at 500 ppm showed greater effects than other treatments. Five hundred ppm calcium hypochlorite and 500 ppm HPAA treatments showed the greatest effects with both 1 and 10 ppm mancozeb after 30 minutes. Figure 48 shows the percent reduction in mancozeb residues in spiked apples at both 3 and 30 minutes. To determine the percent reduction all samples were compared to the control which was exposed only to distilled water. For the 50 ppm chlorine treatment, mancozeb residues were reduced about 56% and 59% at 3 minute and 87% and 75% after 30 minute dipping time in 1 ppm and 10 ppm mancozeb, respectively. For the 500 ppm chlorine and 500 ppm HPAA experiments, most residues were degraded up to 99% in both 1 ppm and 10 ppm mancozeb levels. Ozone at 3 ppm also showed effectiveness in reducing mancozeb levels at 10 ppm level after 30 minutes. Chlorine dioxide at both 5 and 10 ppm showed less effectiveness compared to other treatments. Generally, increased reaction time (30 minutes) reduced mancozeb levels compared to 3 minute reaction time. The overall reaction was much slower and less effective than observed from the solution studies. Degradation of mancozeb at the high concentration (10 ppm) was less effective than at the low concentration (1 ppm).

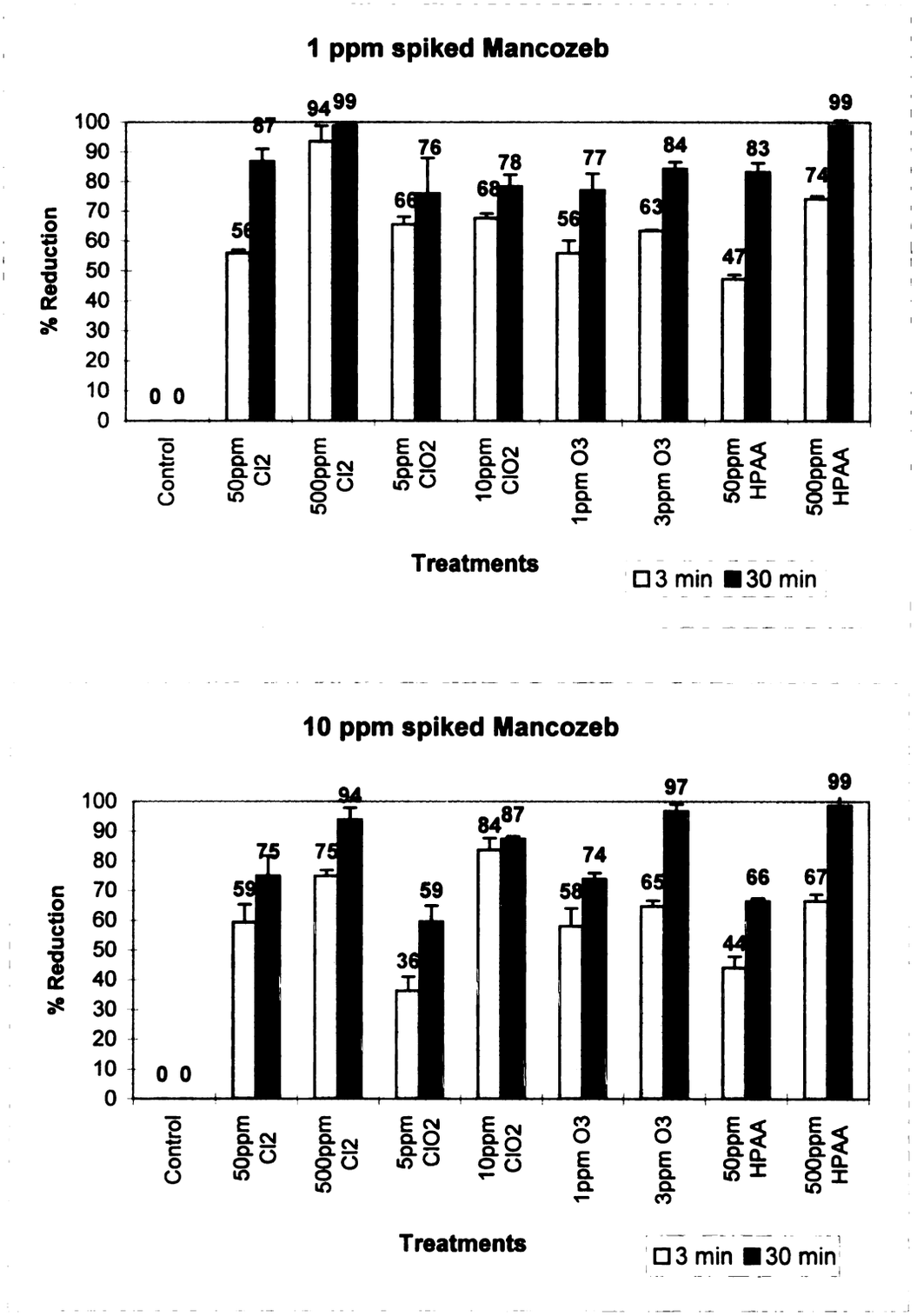
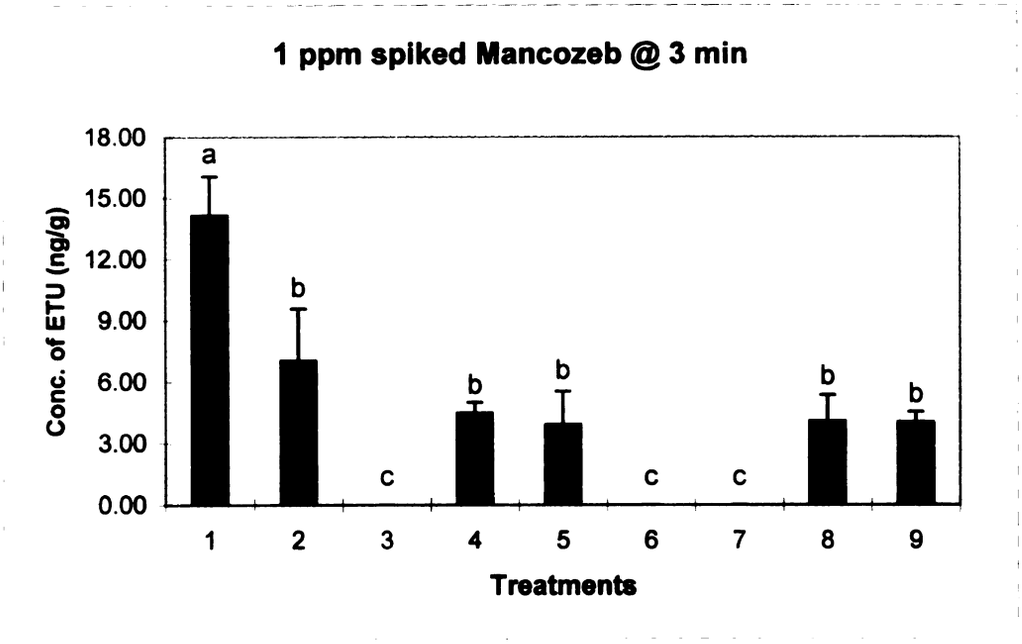


Figure 48. Percent reduction of Mancozeb after various oxidizing agents treatments.

D. Degradation of Mancozeb into ETU in Spiked Apples

The next part of this work was the determination of conversion of mancozeb to ETU in spiked apples. Figures 49–50 and Tables 8–9 shows the ETU residues formed from the 1 and 10 ppm mancozeb spiked apples at 3 and 30 minute reaction times. Mancozeb produced significant quantities of ETU. At the 1 ppm mancozeb, ETU after 3 minutes was 14.13 ppb and slowly increased to 15.12 ppb after 30 minutes reaction time for control which was treated with only distilled water (Figure 49 and Table 8). Various oxidizing agents significantly reduced ETU residue levels compared to the control. For the 1 ppm mancozeb experiments, 500 ppm calcium hypochlorite and 1 and 3 ppm ozone treatments completely inhibited the conversion of mancozeb to ETU (Figure 49). At 3 minutes, chlorine dioxide and HPAA showed powerful effects in reducing ETU levels compared to the control; however, there was no statistical ($p<0.05$) difference between 5 and 10 ppm chlorine dioxide and 50 and 500 ppm HPAA. After 30 minutes, all ETU residues were degraded at high concentrations of the oxidizing agents, small amounts of ETU were still determined at lower concentration of oxidizing agents.

At the 10 ppm mancozeb, the conversion rate of mancozeb into ETU was higher and the oxidizing agent treatments showed less effect than at the 1 ppm level (Figure 50 and Table 9). In this case, increased reaction time and higher concentration of oxidizing agents played an



1. Control
2. 50 ppm chlorine
3. 500 ppm chlorine
4. 5 ppm chlorine dioxide
5. 10 ppm chlorine dioxide
6. 1 ppm ozone
7. 3 ppm ozone
8. 50 ppm HPAA
9. 500 ppm HPAA

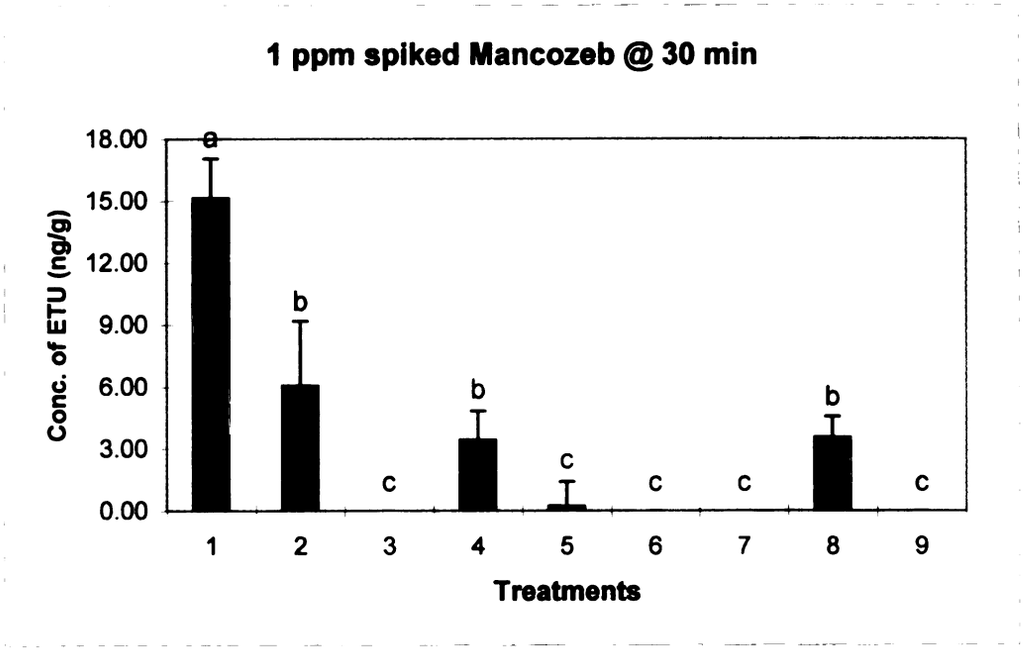
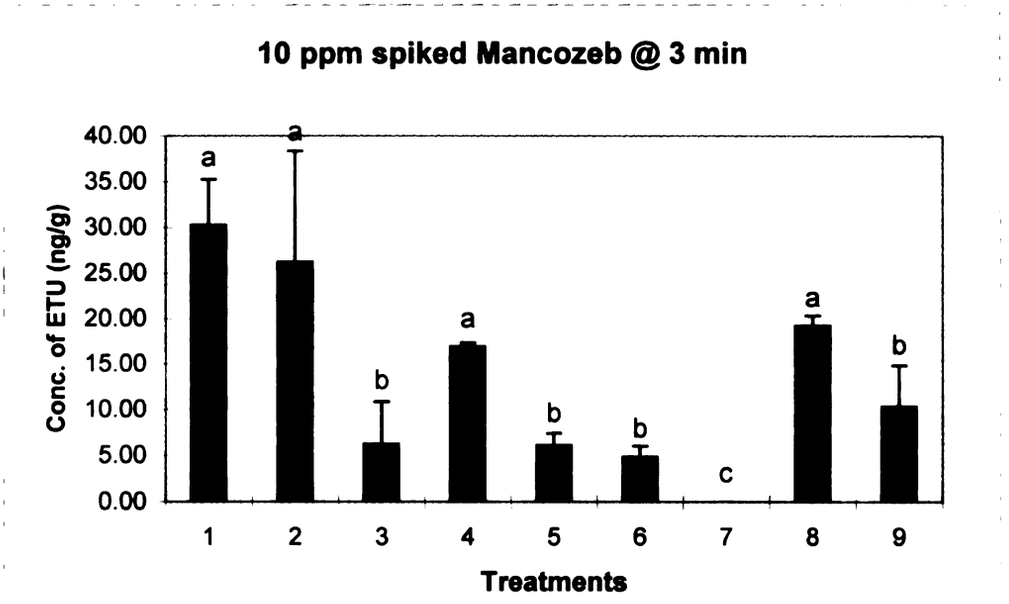


Figure 49. Comparison of various oxidizing agents on the conversion of 1 ppm Mancozeb into ETU.



1. Control
2. 50 ppm chlorine
3. 500 ppm chlorine
4. 5 ppm chlorine dioxide
5. 10 ppm chlorine dioxide
6. 1 ppm ozone
7. 3 ppm ozone
8. 50 ppm HPAA
9. 500 ppm HPAA

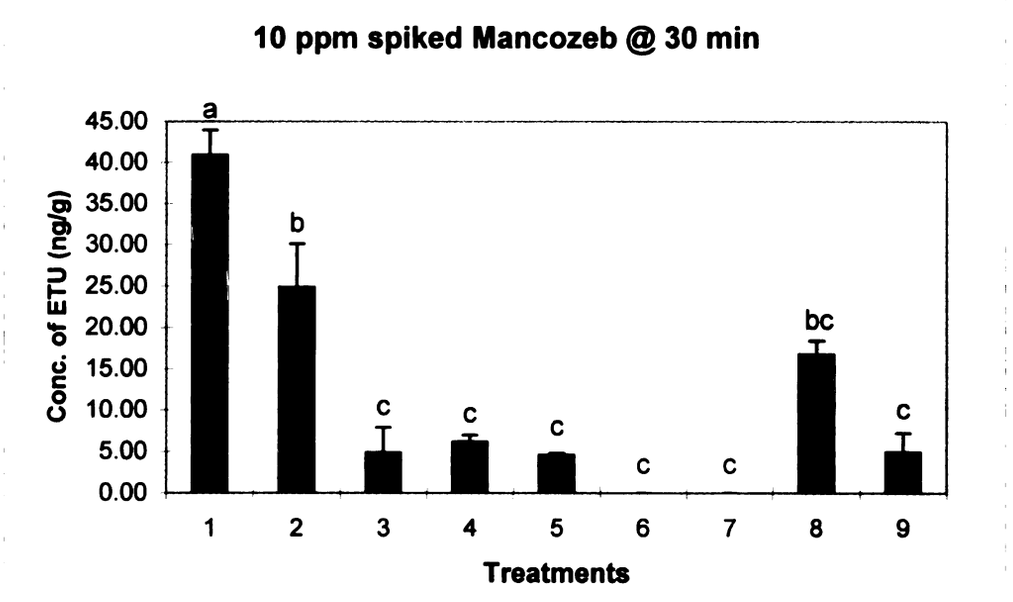


Figure 50. Comparison of various oxidizing agents on the conversion of 10 ppm Mancozeb into ETU.

Table 8. Effects of Various Oxidants on the ETU Residue Concentrations and % Remaining of ETU at 1 ppm Mancozeb Spiked Level

	ETU Conc. (ppb)		% Remaining	
	3 min	**30 min**	**3 min**	**30 min**
Control	14.13 ± 1.93[a]	15.12±6.93[a]	100	100
50 ppm $Ca(OCl)_2$	7.02 ± 2.57[b]	6.04 ±3.14[b]	49.68	39.95
500 ppm $Ca(OCl)_2$	N.D.[c]	N.D[b]	0.00	0.00
5 ppm ClO_2	4.45 ± 0.53 [b]	3.41± 1.42[b]	31.49	2.55
10 ppm ClO_2	3.90 ± 1.63 [b]	0.22± 1.19[b]	27.60	1.46
1ppm O_3	N.D.[c]	N.D.[b]	0.00	0.00
3 ppm O_3	N.D.[c]	N.D.[b]	0.00	0.00
5 ppm HPAA	4.06 ± 1.27[b]	3.55± 1.00[b]	28.73	23.48
50 ppm HPAA	4.00 ± 0.52[b]	N.D.[b]	28.31	0.00

Note: 1. Values are the means of triplicate determinations.

2. Values with same letters are not significantly different ($p<0.05$).

3. N. D. = None Detected

Table 9. Effects of Various Oxidants on the ETU Residue Concentrations and % Remaining of ETU at 10 ppm Mancozeb Spiked Level

	ETU Conc. (ppb)		% Remaining	
	3 min	**30 min**	**3 min**	**30 min**
Control	30.25 ± 5.01[a]	40.88 ± 3.07[a]	100	100
50 ppm $Ca(OCl)_2$	26.17 ± 12.17[a]	24.82 ± 5.28[b]	86.51	60.71
500 ppm $Ca(OCl)_2$	6.23 ± 4.64[b]	4.86 ± 3.02[c]	20.60	11.89
5 ppm ClO_2	16.91 ± 0.44[a]	6.15 ± 0.21[c]	55.90	15.04
10 ppm ClO_2	6.09 ± 1.32[b]	4.57 ± 0.23[c]	20.13	11.18
1 ppm O_3	4.84 ± 1.17[b]	N.D.[c]	16.00	0.00
3 ppm O_3	N.D.[c]	N.D.[c]	0.00	0.00
5 ppm HPAA	19.20 ± 1.10[a]	16.69 ± 1.70[d]	63.47	40.83
50 ppm HPAA	10.34 ± 4.48[b]	4.92 ± 2.35[c]	34.18	12.04

Note: 1. Values are the means of triplicate determinations.

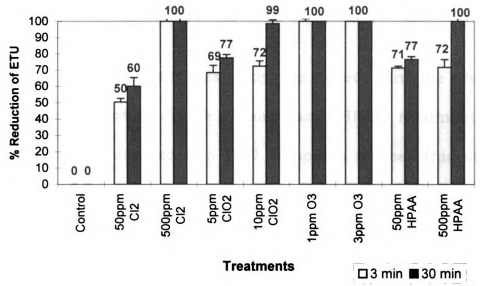
2. Values with same letters are not significantly different ($p<0.05$).

3. N. D. = None Detected

important role in the reduction of ETU residues. Ozone at 3 ppm still showed a powerful effect in reducing ETU levels. Ozone was also very effective in the degradation of mancozeb as compared to other oxidants at low concentration. This is probably due to the high oxidation potential of ozone (2.07 V).

Figure 51 shows the percent reduction of ETU under various oxidizing agent treatments. At 50 ppm calcium hypochlorite, ETU residue decreased up to 50% of initial concentration in 1 ppm spiked mancozeb after 3 minutes. However, at 10 ppm mancozeb level, very little reduction of ETU occurred with only 13 and 39% reduction at 3 and 30 minutes, respectively. This is due to the low concentration of chlorine and high concentration of mancozeb. In 1 and 3 ppm ozone, ETU residues were completely degraded at both 1 ppm and 10 ppm mancozeb. At low mancozeb levels of 1 ppm, chlorine at 500 ppm and HPAA at 500 ppm showed the best effects. However, increased mancozeb concentration decreased the degrade time effects.

These findings indicate that EBDC content of a food should be of concern in addition to ETU residues on the raw agricultural commodities for any realistic evaluation of ETU exposure.



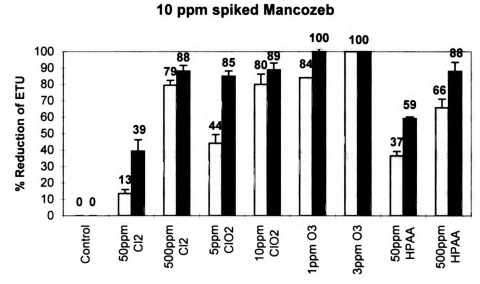


Figure 51. Percent reduction of ETU after various oxidizing agents treatments.

SUMMARY & CONCLUSION

The objective of this study was to determine the effectiveness of chlorine, chlorine dioxide, ozone and HPAA treatment on the degradation of mancozeb and ETU in spiked apples. This study was developed based on model system experiments at ambient pH and temperature. Two different levels of mancozeb (1 and 10 μg/ml) were added to non–mancozeb treated apples. Various oxidizing treatments were effective in reducing or removing ETU residues as well as mancozeb on spiked apples. Mancozeb residues decreased 56–99% with chlorine and 36–87% with chlorine dioxide treatments. In this study, chlorine dioxide showed less effectiveness in mancozeb degradation compared to model study. This was due to low concentration of chlorine dioxide compared to high mancozeb residue. The residue levels would be reduced considerably if the concentration of chlorine dioxide is increased above the 10 ppm that was used in this study. ETU was completely degraded by 500 ppm calcium hypochlorite and 10 ppm chlorine dioxide at 1 ppm spiked level. However, at 10 ppm spiked level, the effectiveness of ETU degradation was lower than observed in 1 ppm spiked level. Mancozeb residues decreased 56–97% with ozone treatment. At 1 and 3 ppm ozone treatment, no ETU residue was detected at 1 ppm spiked mancozeb after

both 3 and 30 minutes. Again, at 10 ppm mancozeb spiked level, the rate of degradation was low. HPAA was also effective in degrading the mancozeb residues with 44–99% reduction at different time and concentrations. ETU was completely degraded at 500 ppm HPAA after 30 minute reaction time.

Studies on whole fruit spiked with mancozeb gave results similar to those found in the model system studies. However, the reaction was much slower than observed for the model systems. These treatments indicated good potential for the removal of pesticide residues on fruit and in processed products.

CHAPTER III. STUDIES ON THE DEGRADATION OF PESTICIDES IN FRESH AND PROCESSED APPLE PRODUCTS

INTRODUCTION

Pesticides are used worldwide to protect crops by controlling insects, diseases, fungi and other pests. Protecting crops from pests gives higher yields, resulting in greater variety and availability of food at a low cost. The demand by consumers for produce with good sensory quality has continued to sustain the use of pesticides for control of insects and diseases in fruits and vegetables. However, along with the benefits there are potential effects of trace amounts of residues remaining on some fruits and vegetables. Consumer groups have expressed concerns about food safety, especially, pesticide residues on (or in) produce at harvest. As a result, there is a need to develop methods for removing or reducing the pesticide residues on fresh and processed fruits and vegetables after harvest. Such methods could alleviate concerns about the hazard of pesticide residues to humans and environment. Postharvest treatments, such as the postharvest water wash and scrub that have been traditionally employed to remove debris and dirt, have been shown to reduce pesticide residues (El Hadidi, 1993). The use of postharvest chlorine dips and ozonated water dips shows potential effect in the removal of pesticide residues (Hendrix, 1991; Ong, 1996). Chlorination and ozonation which are the principal processes of water purification,

may produce by-products as a result of reaction between chlorine or ozone and pesticides in raw material, and it has been reported that for some organophosphate pesticides, the degradation by–products have higher toxicity than the original pesticides themselves (Kobayashi *et al.*, 1990). Chlorine dioxide has been used since 1944 by the food industry as a sanitizing and disinfecting agent and for oxidizing organic compounds at water treatment plants. It has a greater oxidizing capacity than chlorine and does not react with ammonia or nitrogenous compounds like chlorine (Bohner and Bradley, 1991). The use of a mixture of acetic acid, hydrogen peroxide and peroxyacetic acid has also been considered as a postharvest treatment method.

Apples (*Malus X domestica* Borkh.) are considered to be a major agricultural product in Michigan (Downing, 1989). Michigan is one of the nation's most important apple producing states, with approximately 9% of the total U.S. production (Ricks and Hull, 1992). As a result of its high economic value as well as the large number of plant disease, insects, and mites that infest apples during their growing seasons, significant quantities of pesticides are often necessary for the protection of this crop. This leads to residues on (or in) the fruit at harvest. The most widespread apple disease, accounting for much of the apple pesticide use worldwide, is apple scab, caused by the fungus *Venturia inaequalis* (Merwin *et al.*, 1994)

The pesticide selected in this study was mancozeb (dithane 75 DF®), which is an ethylenebisdithiocarbamate (EBDC). EBDCs are fungicides which are frequently used for the control of fungal diseases in a wide range of fruits and vegetables. These substances are not stable in the presence of moisture, oxygen, and in biological systems. During their degradation, several products are formed, but ethylenethiourea (ETU) is one of the major by–products. Many researchers are interested in ETU because this compound has been found to be carcinogenic and teratogenic for laboratory animals and is considered dangerous to human health (Fishbein, 1976).

The previous solution laboratory studies and the whole fruit studies were used to determine the optimum parameters for this orchard study. The objectives of this study are 1) reduce or eliminate mancozeb and ETU residues in apples and apple products; 2) determine the effectiveness of different post harvest treatments and processing on the reduction of mancozeb and ETU residues when treated with chlorine, chlorine dioxide, ozone or hydrogen peroxyacetic acid.

MATERIALS AND METHODS

MATERIALS

A. Apple Samples

Mature Cortland apples (1997) and Golden Delicious apples (1998 and 1999) were harvested from the Botany Research Field Laboratory at Michigan State University, East Lansing, at various preharvest intervals. The fruits were hand picked randomly from various regions of the treated trees, thoroughly mixed and stored at 4°C until they were processed for residue analysis.

B. Reagents

(I) Solvents

All organic solvents used for preparation of stock solution, in sample extraction and high performance liquid chromatography (HPLC) were distilled-in-glass grade. Acetone and methylene chloride were obtained from J. T. Baker, Co. (Phillipsburg, NJ).

(II) Chemicals

Mancozeb standard was obtained from Rohm & Haas (Philadelphia, PA). ETU standard was obtained from Aldrich Co. (Milwaukee, WI). The standard stock solutions of mancozeb and ETU were prepared in distilled water at concentration of 100 μg/100 ml. The standards were protected from light and stored in refrigerator. Chlorine solutions were prepared from calcium hypochlorite (Aldrich, Milwaukee, WI) as a source of chlorine. Chlorine dioxide was generated in the laboratory using the manufacturer's (S.C. Johnson Professional, WI.) instructions. Hydrogen peroxyacetic acid stock solution (Ecolab Inc.) were used as a source of hydrogen peroxide. Sodium thiosulfate, sodium sulfate, potassium iodide, potassium indigo trisulfonate were all reagent grade.

C. Glassware

All glassware was thoroughly washed with detergent and warm water then rinsed with distilled water. The glassware was then rinsed with acetone and placed in an oven at 400°C overnight before use.

METHODS

The solution studies and the whole fruit studies were used to determine the optimum parameters for this orchard study. The orchard study was set up to investigate the effect of various postharvest wash treatments and the effects of processing on the reduction of mancozeb and ETU residues under actual field conditions.

A. Pesticide Application and Spray Schedule

The orchard study was conducted during three years (1997–1999) and two different varieties were used; i) Cortland (1997) and ii) Golden Delicious (1998 and 1999). Apples were grown at the Botany Research Field Laboratory at Michigan State University in East Lansing. The field diagrams are shown in Appendix 10. Maintenance pesticides were applied throughout the growing season to provide the necessary insect, mite and disease control. All pesticides were applied as a foliar spray with an FMC airblast sprayer at 80 gallons/acre and 300 psi.

Seventy seven day preharvest intervals (PHI) for 1997 studies and 4–day PHI for 1998 studies were used. In the case of 1997 studies, the mancozeb residues in some apple products were below detectable limits (BDL), so, 4–day PHI was used in the 1998 studies to ensure the measurable amount of residue on the fruits. For the final application,

mancozeb was tank-mixed and applied as a single application. A PHI study was conducted in 1999. The apples were harvested at of PHI 0, 1, 4, 7, 14 and 77 day along with a control. Control samples did not receive a final application. Tables 10–12 show the records for the 1997–1999 spray applications.

B. Apple Sampling and Harvesting

At optimum harvest maturity the apples were randomly hand picked from all regions of the tree except fruit within one foot of the ground which was excluded. Gloves were worn during harvest and changed between treatments and control to prevent cross contamination. Twelve crates (53–60 lbs/crate) of fruit were collected from each of the areas. Immediately following harvest, the samples were transported to refrigerated cubicles (2–4°C) for storage at the MSU Food Science and Human Nutrition building until postharvest treatment and processing.

Control samples produced in 1998 were found to contain residues of mancozeb. This was due to the cross contamination during spraying. Thus control samples were obtained from a commercial orchard which had not been sprayed with mancozeb in 1998. Refer to Table 13 for records of the spray schedule of the sample. Control apples received the same treatments and processes as described for the treated samples.

Table 10. 1997 Spray Schedule for Cortland Apples (77 day PHI)

Dates	Chemicals	Rate	Purpose of Application	Temp.
April 21	Dithane 75DF	3 lb/A	treatment spray	40–45°F
	Nova 40W	5 oz/A	apple scab	
April 28	Dithane 75DF	3 lb/A	treatment spray	40°F
	Nova 40W	5 oz/A	apple scab	
May 7	Dithane 75DF	3 lb/A	treatment spray	60°F
	Nova 40W	5 oz/A	apple scab	
May 16	Dithane 75DF	3 lb/A	treatment spray	35°F
	Nova 40W	5 oz/A	apple scab	
May 27	Dithane 75DF	3 lb/A	treatment spray	40°F
	Nova 40W	5 oz/A	apple scab	
June 6	Dithane 75DF	3 lb/A	treatment spray	60°F
	Nova 40W	5 oz/A	apple scab	
June 16	Dithane 75DF	3 lb/A	treatment spray	74°F
	Nova 40W	5 oz/A	apple scab	
June 30	Dithane 75DF	3 lb/A	treatment spray	75°F
	Nova 40W	5 oz/A	apple scab	
July 14	Guthion 50W	1.5 lb/A	curculio, aphids	75°F
July 24	Guthion 50W	1 lb/A	curculio, aphids	70°F

Note – 1. Dithane 75 DF: Trade name of Mancozeb
2. 3 lb/acre is equivalent to 3.36 kg/ha.
2. Control spray: Nova 40W + Captan 50W
3. Harvest date: September 15, 1997 (1:30-2:40 PM)

Table 11. 1998 Spray Schedule for Golden Delicious Apples (4 day PHI)

Dates	Chemicals	Rate	Purpose of Application	Temp.
April 1	Dithane 80DF	3 lb/A	treatment spray	50–55°F
	Nova 40W	5 oz/A	apple scab	
April 7	Dithane 80DF	3 lb/A	treatment spray	35–40°F
	Nova 40W	5 oz/A	apple scab	
April 14	Dithane 80DF	3 lb/A	treatment spray	45–50°F
	Nova 40W	5 oz/A	apple scab	
April 21	Dithane 80DF	3 lb/A	treatment spray	60°F
	Nova 40W	5 oz/A	apple scab	
April 28	Dithane 80DF	3 lb/A	treatment spray	35–40°F
	Nova 40W	5 oz/A	apple scab	
May 6	Dithane 80DF	3 lb/A	treatment spray	50°F
	Nova 40W	5 oz/A	apple scab	
May 15	Dithane 80DF	3 lb/A	treatment spray	70°F
	Nova 40W	5 oz/A	apple scab	
June 1	Dithane 80DF	3 lb/A	treatment spray	65°F
	Nova 40W	5 oz/A	apple scab	
	Guthion 50W	1 lb/A	curculio, aphids	
June 15	Dithane 80DF	3 lb/A	treatment spray	50–60°F
	Nova 40W	5 oz/A	apple scab	
	Guthion 50W	1 lb/A	curculio, aphids	
June 29	Dithane 80DF	3 lb/A	treatment spray	80°F
	Nova 40W	5 oz/A	apple scab	
	Guthion 50W	1 lb/A	curculio, aphids	
	Pyramite	4.4oz/A		
July 16	Dithane 80DF	3 lb/A	treatment spray	70–75°F
	Nova 40W	5 oz/A	apple scab	
	Guthion 50W	1 lb/A	curculio, aphids	
Sep. 28	Dithane 75 DF	3 lb/A	treatment spray	60°F

Note – 1. Dithane 80DF and Dithane 75 DF: Trade name of Mancozeb
2. 3 lb/acre is equivalent to 3.36 kg/ha.
3. Control spray: Nova 40W + Captan 50W
4. Harvest date: October 2, 1998 (8:00-9:30 AM)

Table 12. 1999 Spray Schedule for Golden Delicious Apples

Dates	Chemicals	Rate	Purpose of Application	Temp.
April 13	Dithane 75DF	3 lb/A	treatment spray	32°F
	Nova 40W	5 oz/A	apple scab	
April 20	Dithane 75DF	3 lb/A	treatment spray	40–45°F
	Nova 40W	5 oz/A	apple scab	
April 26	Dithane 75DF	3 lb/A	treatment spray	40°F
	Nova 40W	5 oz/A	apple scab	
May 5	Dithane 75DF	3 lb/A	treatment spray	50°F
	Nova 40W	5 oz/A	apple scab	
May 11	Dithane 75DF	3 lb/A	treatment spray	55–60°F
	Nova 40W	5 oz/A	apple scab	
May 28	Dithane 75DF	3 lb/A	treatment spray	60°F
	Nova 40W	5 oz/A	apple scab	
June 14	Dithane 75DF	3 lb/A	treatment spray	60°F
	Nova 40W	5 oz/A	apple scab	
June 28	Dithane 75DF	3 lb/A	treatment spray	70°F
	Nova 40W	5 oz/A	apple scab	
July 12	Dithane 75DF	3 lb/A	treatment spray	65°F
	Nova 40W	5 oz/A	apple scab	
July 22	Dithane 75DF	3 lb/A	treatment spray	75°F
	Nova 40W	5 oz/A	apple scab	
August 9	Dithane 75DF	3 lb/A	treatment spray	60–65°F
	Nova 40W	5 oz/A	apple scab	
Sep. 17	Dithane 75DF	3 lb/A	treatment spray	55–55°F
	Nova 40W	5 oz/A	apple scab	

Note – 1. Dithane 75 DF: Trade name of Mancozeb
2. 3 lb/acre is equivalent to 3.36 kg/ha.
3. Control spray: Nova 40W + Captan 50W
4. Sampling date: Sep.17 (0 day PHI) at 10 A.M.
Sep. 18 (1 day PHI) at 10 A.M.
Sep. 21 (4 day PHI) at 10 A.M.
Sep. 24 (7 day PHI) at 10 A.M.
Oct. 1 (14 day PHI) at 10 A.M.
Oct. 25 (77 day PHI) at 10 A.M.

Table 13. 1998 Spray Schedule for Golden Delicious Apples (Control)

Dates	Chemical	Purpose
April 27	Syllit	Scab
May 8	Syllit	Scab
May 10	Captan	Scab

* After May 10, spraying was aborted due to hail damage.

C. Fruit Postharvest Treatments

All postharvest treatment and processing were conducted at the Fruit and Vegetable Processing Laboratory, Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI. Batches of apples (1.5–2 lbs) from each of the sample groups were subjected to postharvest treatments, processed and then analyzed for mancozeb and ETU residues in whole apples, peeled and cored slices, peeled and cored sauce, unpeeled and uncored sauce, juice and wet pomace.

(I) Wash Treatments

All wash treatments were prepared in 20–liter containers with 7 liters of wash solution to allow for complete submersion of the apple samples. 1.5–2 lbs apples were used per replication (3 replications per

treatment) and the use of mesh bags allowed samples to be easily removed after 15 minute wash time. The three treatments in 1997 were: (1) No wash, (2) Water wash and (3) Calcium hypochlorite wash @ 50 and 500 ppm. The six treatments in 1998 were: (1) No wash, (2) Water wash, (3) Calcium hypochlorite wash @ 50 and 500 ppm, (4) Chlorine dioxide wash @ 10 ppm, (5) Ozone wash @ 3 ppm and (6) HPAA wash @ 50 ppm. The temperature, pH, chorine, chlorine dioxide, ozone and HPAA concentration were monitored before and after each wash treatment. A calibrated pH meter, model 601A (Corning Glass Works, Medfield, MA), was used to determine the pH of the wash treatments.

(II) Sample Processing

Fruit from all plots, replication and treatments were processed into peeled and cored slices, peeled and cored apple sauce, unpeeled and uncored apple sauce, juice and pomace. The methods used were Standard Operating Procedures developed by the Department of Food Science and Human Nutrition, Michigan State University. Apples were postharvest-treated and/or processed within one week of harvest. All samples were weighed before and after processing in order to obtain percent yield. The controls were treated and processed before the mancozeb treated samples. After each sample was processed, the

processing equipment was thoroughly cleaned and pressure washed to prevent cross contamination of samples with mancozeb residues.

1. Slices

Apples for slices were weighed, then peeled and cored on a Leader apple peeler. The peeled and cored apples were sliced with a Sunkist slicer. Samples for slices were weighed and placed into labeled plastic ziplock bags then sealed and frozen at –20°C storage immediately to await residue analysis.

2. Sauce, Peeled and Unpeeled

Two–lb samples of apples were processed into apple sauce (peeled-cored and also unpeeled–uncored). The apples were first sliced with a Sunkist slicer and subsequently blanched in a Dixie steam blancher for 10 minutes at approximately 110°C to adequately soften the fruit and inactivate enzymes. After steaming, the apples were cooled in air for 1 to 2 minutes and then were passed through a Langsencamp pilot plant finisher with a 0.033–0.045 inch screen to remove coarse fibers, seeds, stems, and peel particles. The apple sauce samples were transferred into plastic ziplock bags immediately after finishing, weighed and stored at –20°C for residue analysis.

3. Juice

One batch of apples was processed into juice. Weighed, unpeeled apples were sliced with a Sunkist slicer. The apple slices were macerated in an Acme Juicerator equipped with stainless steel blades. The grinder/centrifuge basket was lined with one layer of Kimwipe tissue. Apple slices were introduced into the juicerator and were automatically ground, juiced and filtered. The volume of juice was measured, filled into French square glass bottles and frozen at –20°C immediately to await residue analysis.

4. Pomace

Pomace is the by-product of juice processing. After juice processing, the pomace was collected, placed in labeled plastic ziplock bags, and immediately frozen (–20°C) until analysis.

D. Pesticide Residue Analyses

(I) Mancozeb

Mancozeb residues were analyzed as carbon disulfide (CS_2) by gas liquid chromatographic headspace analysis (Ahmad *et al*, 1995). Twenty mls of sample were transferred at 0, 5, 15, and 30 minute intervals into sample bottles. A 0.5% 0.1 M sodium thiosulfate solution was added to the samples at the appropriate time to quench the reaction.

Forty mls of 1.5% stannous chloride in 5 M HCl were added and immediately sealed with a crimped septum. Fifty µls of a 1mg/ml thiophene solution were injected into each bottle and incubated at 70–80°C in a water bath for 15 minutes. Bottles were removed and agitated for 2 minutes by hand. Bottles were replaced in the water bath with repeated shaking for 1 hour. A 100 µl sample was removed with a gas tight syringe from the bottle headspace and injected into the GC.

(II) ETU

ETU residues were determined using a modification of the HPLC method published by Ahmad *et al.* (1995). Twenty mls of sample were weighed into blender cup and added 160 ml of methanol + water (3:1, V/V) blended with a homogenizer for 3 minutes at high speed. The slurry was filtered under vacuum through a buchner funnel with a #4 Whatman filter paper. The blender cup and solids on filter were mixed with 30 ml of solvent mixture. These were combined with initial filtrate into Turbovap tube and concentrated extract at 60°C to ca. 20 ml on a Zymark Turbovap evaporator (Zymark Inc., Hopkin, MA). The extracts were quantitatively transferred to an Erlenmeyer flask by rinsing tubes with 5 ml distilled water, and then 8 g of potassium fluoride and 0.6 g of ammonium chloride were added. This mixture was extracted with 50 ml methylene chloride two times. The methylene chloride layer was passed

through a bed of 25 g anhydrous sodium sulfate, collected in a Zymark Turbovap tube and evaporated to dryness on an automated Zymark Turbovap evaporator at 40°C. The residue was dissolved in 3 ml distilled water and 75 µls were injected into an HPLC column.

(III) Recovery Study

To evaluate performance of the foregoing analytical procedures, known amounts of different concentration levels of mancozeb and ETU were added to samples. Recoveries (% recovery) were determined at fortification levels ranging from 0.01 to 2 ppm for mancozeb and 0.005 to 2 ppm for ETU and the fortified samples were then carried through the above procedures. Percent recovery was derived from the equation:

$$\% \text{ Recovery} = \frac{\text{Amount of pesticide obtained}}{\text{Amount of pesticide added}} \times 100$$

E. Chromatographic Analyses

(I) Mancozeb Residue Analyses

Mancozeb residues were detected and quantified using a Hewlett Packard Series II 5890 gas chromatograph (GC) equipped with a flame photometric detector (FPD) in the sulfur mode. The GC was equipped with a Supel–Q–Plot fused silica capillary column (30 m long x 0.53 mm ID) with a film thickness of 0.25 µm (Supelco Inc., Bellefonte, PA). The oven temperature was 80°C, while the injector and detector

temperatures were 230°C and 300°C, respectively. Helium and nitrogen were used as the GC carrier gas and makeup gas, respectively. Carrier gas flow through the column was 20ml/min. Integration was carried out with HP Chemstation software interfaced to the GC.

(II) ETU Residue Analyses

A liquid chromatograph with a Hypersil BDS C_{18} column (250 mm x 4.6 mm, 5 μm particles), a Hypersil BDS C_{18} guard column (10 mm x 4.6 mm, 5 μm particles) UV detector set at 240 nm were used. The mobile phase was 0.72% butylamine in distilled water at pH 3.0–3.2. A M–45 Waters HPLC pump (Waters Associates, Inc., Milford, MA.) was used for solvent delivery at a flow rate of 0.5 ml/minutes. After the system was stabilized (about 1 hour from initial warm–up), 75 μl samples were injected via Rheodyne syringe loop injector (50 μl loop) for analysis. Integration was carried out using 3390 A Hewlett Packard integrator.

F. Calculation of Pesticide Residue Concentration

Mancozeb and ETU residue concentrations in fresh or processed apples were calculated based on the area of the integrated peaks of the samples compared with known concentrations of analytical standard of the respective pesticides. Standard curves of the mancozeb and ETU were plotted and least square linear regression were obtained using

Microsoft Excel (Microsoft Corporation, Redmond, WA) software. Examples of standard curves for mancozeb and ETU standard of known concentrations are provided in the appendix.

The residue concentrations were calculated based on the following formula:

(a) Mancozeb residue in μg/ml

$$\text{ppm} = \frac{\text{ng Mancozeb}}{\text{mg sample injected}}$$

where, ng Mancozeb was derived from standard curve

$$\text{mg sample injected} = \frac{20\text{g}}{\text{headspace volume sample – containing reaction vial} \times \mu\text{L injected}}$$

where, headspace volume of sample - containing reaction vial = 40 mL

(b) ETU residue in μg/ml

$$\frac{\text{Conc. of ETU in sample extract based on std. curve}(\mu\text{g/g}) \times \text{Vol. final extract (3 ml)}}{\text{Weight of sample analyzed (20 g)}}$$

G. Statistical Analysis

All determinations were replicated three times. Mean standard deviations, mean square errors, two factor ANOVA, correlation and

interaction of main effects were calculated using Sigmastat computer software 1.0 (Jandel Corp., San Rafael, CA). Appropriate comparisons were made using Student–Newman–Keuls Method for multiple comparisons. A $p<0.05$ was considered statistically significant.

RESULTS & DISCUSSION

A. Pesticide Residues in Unprocessed Apples

Two different varieties of apples were used in this study. Table 14 shows the comparison of specific characteristics of Cortland and Golden Delicious apples. These are dual-purpose apple cultivars, for fresh eating and processing (Downing, 1989). The reason for using two different varieties of apples was to determine the relationship between surface waxes and pesticide degradation patterns. However, no specific relationships were noted for these characteristics between the two cultivars from these experiments.

(I) Effect of PHI on the Pesticide Residue Levels

Preharvest interval (PHI), which is the time interval between the last spray application and harvest, has been shown to affect pesticide residue levels. Figure 52 shows the rate of decline for ETU on apples according to PHI. The points on the curves represent an average of three determinations. ETU residue concentration on the day of application was approximately 2.47 ppm. The residue slightly increased after 1 day PHI up to 2.50 ppm. However, this decreased gradually to about 1.89 ppm,

Table 14. Comparison of specific characteristics of Cortland and Golden Delicious Apples

Name	Size	Skin Color	Flesh Color	Texture	Flavor	Note
Cortland	medium	red stripes over yellow green	white	coarse, juicy	sweet tart but not intense	good for salad, but makes a poorly colored sauce
Golden Delicious	medium to medium large	golden yellow (often russet due to cool weather)	bright light yellow	juicy, firm	sweet and sprtiely	all purpose; preferred for processing because the russet makes unpleasant appearance

(Way and McLellan, 1989; Manhart, 1995)

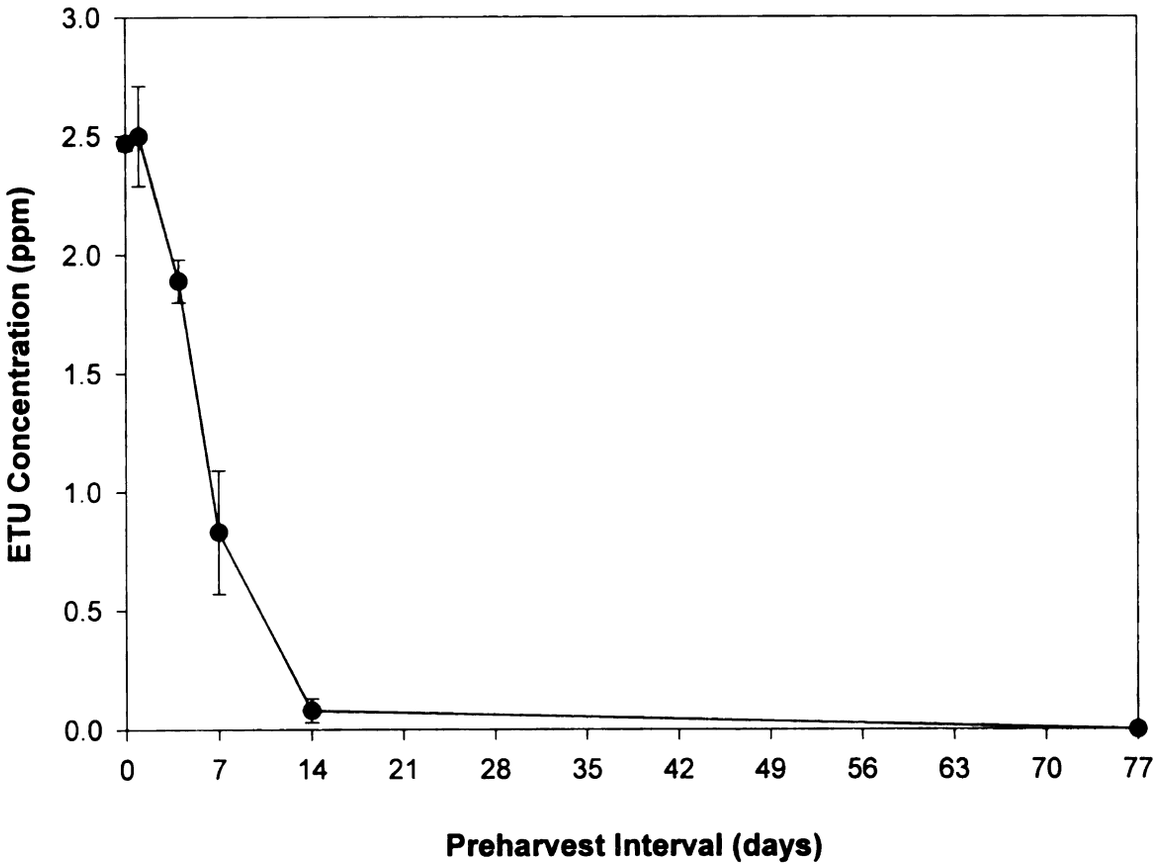


Figure 52. Effect of PHI on ETU residues in raw apples.

0.84 ppm, 0.07 ppm after 4, 7 and 14 days, respectively. There was no indication that ETU accumulated in the apples at 77 days. There were several heavy rains between the 3 and 14 day. This seemed to cause major changes in ETU residue concentrations.

Various studies indicate that longer PHI's help to lower pesticide residue levels. El–Zamity (1988) and Rashid *et al.* (1987) both reported lower residue levels for captan, with an increase in PHI, on tomatoes and apples, respectively. Similar studies by El–Hadidi (1993) and Belanger *et al.* (1991) found residue levels to decrease with increased PHI. Pesticide manufacturers often recommend a specific PHI in combination with a particular crop and pesticide. Such recommendations help to reduce the chance of residues exceeding federal tolerances. The recommended PHI for mancozeb (Dithane 75 DF®) is 77 days for apple (Code of Federal Regulations, 1996). For the first year (1997) studies, 77 days PHI was applied. In this case, there was no evidence of ETU residue in apples and apple products. Analysis of these samples indicated ETU residues to be absent or below the method of detection limit.

(II) Apple Processing & Product Yield

The apples were processed into slices, unpeeled sauce, peeled sauce, juice and wet pomace. Tables 15–16 show yield data for two years

Table 15. Percent Yields of Processed Apple Products, 1997

Product	Wash Treatment	Raw Apple Wt.(g)	Product Wt. (g)	Yield (%)
Whole Fruit	No Wash	831 ± 70	831 ± 70	100.00 ± 0.00
	Water Wash	820 ± 10	820 ± 10	100.00 ± 0.00
	$Ca(OCl)_2$ @ 50 ppm	793 ± 50	793 ± 50	100.00 ± 0.00
	$Ca(OCl)_2$ @ 500 ppm	829 ± 19	829 ± 19	100.00 ± 0.00
Slice	No Wash	835 ± 59	630 ± 73	75.45 ± 8.77
	Water Wash	823 ± 5	660 ± 30	80.14 ± 3.10
	$Ca(OCl)_2$ @ 50 ppm	817 ± 47	648 ± 45	75.45 ± 1.04
	$Ca(OCl)_2$ @ 500 ppm	811 ± 10	621 ± 43	76.50 ± 4.39
Unpeeled Sauce	No Wash	838 ± 34	317 ± 31	37.79 ± 3.40
	Water Wash	823 ± 37	384 ± 50	46.82 ± 7.40
	$Ca(OCl)_2$ @ 50 ppm	775 ± 27	318 ± 21	41.05 ± 3.23
	$Ca(OCl)_2$ @ 500 ppm	817 ± 16	344 ± 43	42.05 ± 4.44

* Values are the means of triplicate determinations.

Table 15 (cont'd)

Product	Wash Treatment	Raw Apple Wt.(g)	Product Wt. (g)	Yield (%)
Peeled Sauce	No Wash	829 ± 14	245 ± 16	29.57 ± 2.29
	Water Wash	799 ± 36	203 ± 25	25.36 ± 2.74
	$Ca(OCl)_2$@ 50 ppm	841 ± 37	236 ± 16	28.13 ± 2.95
	$Ca(OCl)_2$@ 500 ppm	832 ± 37	209 ± 12	25.07 ± 0.69
Juice	No Wash	817 ± 50	219 ± 47	26.75 ± 4.55
	Water Wash	844 ± 37	261 ± 13	31.03 ± 2.84
	$Ca(OCl)_2$ @ 50 ppm	817 ± 55	251 ± 19	30.70 ± 0.67
	$Ca(OCl)_2$ @ 500 ppm	805 ± 21	246 ± 7	30.60 ± 0.22
Pomace	No Wash	817 ± 50	175 ± 28	21.39 ± 2.16
	Water Wash	844 ± 37	197 ± 22	23.36 ± 3.11
	$Ca(OCl)_2$ @ 50 ppm	817 ± 55	194 ± 16	23.74 ± 1.22
	$Ca(OCl)_2$@ 500 ppm	805 ± 21	191 ± 18	23.72 ± 2.05

* Values are the means of triplicate determinations.

Table 16. Percent Yields of Processed Apple Products, 1998

Product	Wash Treatment	Raw Apple Wt.(g)	Product Wt. (g)	Yield (%)
Whole Fruit	No Wash	974 ± 26	974 ± 26	100.00 ± 0.00
	Water Wash	981 ± 19	981 ± 19	100.00 ± 0.00
	$Ca(OCl)_2$ @ 50 ppm	702 ± 21	702 ± 21	100.00 ± 0.00
	$Ca(OCl)_2$ @ 500 ppm	929 ± 23	929 ± 23	100.00 ± 0.00
	ClO_2 @ 10 ppm	784 ± 35	784 ± 35	100.00 ± 0.00
	O_3 @ 3 ppm	880 ± 27	880 ± 27	100.00 ± 0.00
	HPAA @ 50 ppm	854 ± 46	854 ± 46	100.00 ± 0.00
Slice	No Wash	1005 ± 39	772 ± 8	75.45 ± 2.28
	Water Wash	953 ± 31	745 ± 33	78.10 ± 1.24
	$Ca(OCl)_2$ @ 50 ppm	684 ± 44	490 ± 33	75.45 ± 1.88
	$Ca(OCl)_2$ @ 500 ppm	896 ± 29	651 ± 19	72.65 ± 1.44
	ClO_2 @ 10 ppm	742 ± 18	478 ± 14	64.35 ± 1.09
	O_3 @ 3 ppm	823 ± 26	590 ± 24	71.65 ± 1.42
	HPAA @ 50 ppm	778 ± 32	548 ± 27	70.41 ± 0.78

* Values are the means of triplicate determinations.

Table 16 (cont'd)

Product	Wash Treatment	Raw Apple Wt.(g)	Product Wt. (g)	Yield (%)
Unpeeled	No Wash	996 ± 17	442 ± 26	44.38 ± 2.20
Sauce	Water Wash	912 ± 25	393 ± 10	43.12 ± 0.52
	$Ca(OCl)_2$ @ 50 ppm	651 ± 27	308 ± 47	47.31 ± 5.25
	$Ca(OCl)_2$ @ 500 ppm	944 ± 23	406 ± 14	42.95 ± 0.86
	ClO_2 @ 10 ppm	808 ± 24	375 ± 14	46.45 ± 0.91
	O_3 @ 3 ppm	805 ± 25	315 ± 37	39.00 ± 3.34
	HPAA @ 50 ppm	841 ± 7	375 ± 14	44.58 ± 1.87
Peeled Sauce	No Wash	977 ± 16	275 ± 10	28.13 ± 0.73
	Water Wash	926 ± 59	248 ± 5	26.87 ± 1.73
	$Ca(OCl)_2$ @ 50 ppm	699 ± 9	185 ± 14	26.43 ± 2.33
	$Ca(OCl)_2$ @ 500 ppm	972 ± 18	291 ± 8	29.92 ± 0.74
	ClO_2 @ 10 ppm	748 ± 5	230 ± 31	30.69 ± 3.87
	O_3 @ 3 ppm	832 ± 14	182 ± 14	21.85 ± 1.94
	HPAA @ 50 ppm	830 ± 26	182 ± 13	21.88 ± 0.91

* Values are the means of triplicate determinations.

Table 16 (Cont'd)

Product	Wash Treatment	Raw Apple Wt.(g)	Product Wt. (g)	Yield (%)
Juice	No Wash	983 ± 10	303 ± 18	30.77 ± 1.67
	Water Wash	950 ± 14	286 ± 8	30.10 ± 0.80
	$Ca(OCl)_2$ @ 50 ppm	611 ± 5	188 ± 7	30.71 ± 0.84
	$Ca(OCl)_2$ @ 500 ppm	972 ± 10	297 ± 7	30.53 ± 0.65
	ClO_2 @ 10 ppm	753 ± 24	222 ± 5	29.54 ± 1.42
	O_3 @ 3 ppm	717 ± 28	224 ± 7	31.24 ± 0.89
	HPAA @ 50 ppm	702 ± 10	219 ± 19	31.21 ± 2.33
Pomace	No Wash	983 ± 10	239 ± 23	24.29 ± 2.07
	Water Wash	950 ± 14	227 ± 17	23.90 ± 1.92
	$Ca(OCl)_2$@ 50 ppm	611 ± 5	148 ± 14	24.23 ± 2.31
	$Ca(OCl)_2$ @ 500 ppm	972 ± 10	227 ± 24	23.37 ± 2.53
	ClO_2 @ 10 ppm	753 ± 24	182 ± 18	24.08 ± 1.58
	O_3@ 3 ppm	717 ± 28	163 ± 10	22.79 ± 1.11
	HPAA @ 50 ppm	702 ± 10	154 ± 16	21.96 ± 2.03

* Values are the means of triplicate determinations.

of processing. The yield data shows various differences in final products. This may be a result of a number of factors including condition of the apples (firmness or juiciness), size, shape, and the efficiency of the processing method. From an economic standpoint maximum recovery is desirable. In juice processing, as the season progresses and apples are less firm, the yield decreases even though the amount of press aid and pressing time is increased (Downing, 1989). The low yield was a direct result of processing, coarse fibers, seeds, stems, and peel particles. Peeled apple sauce and pomace showed lower yield than other products because of the number of unit operations necessary to obtain this product.

B. Recovery Study

Recovery studies for mancozeb and ETU were carried out in triplicate using the previously described analytical methods. The recovery samples were prepared by adding a known amount of each pesticide to a 20 g sample of apple products and taking each sample through the entire analytical method. Samples were spiked at a level of 0.2 and 2.0 μg/g for both mancozeb and ETU. Table 17 gives percent recoveries of mancozeb and ETU added to apple products prepared with apples not treated with mancozeb. Recovery percents ranging from 76% to 99.8% are indication of good and dependable analytical procedures. Recoveries in this study

were higher than 80% for all levels except for the lowest ETU addition. The method of detection limit (MDL) for mancozeb and ETU was determined to be 0.01 μg/g and 0.005 μg/g, respectively.

Table 17. Percent Recovery of Mancozeb and ETU

	Apple products	**0.01 μg/g ***	**0.2 μg/g**	**2.0 μg/g**
Mancozeb	Slices	80.4 ± 5.1	89.6 ± 2.9	85.3 ± 4.2
	Unpeeled sauce	77.5 ± 4.6	90.4 ± 4.8	93.4 ± 5.3
	Peeled sauce	82.8 ± 7.6	85.2 ± 5.0	90.5 ± 5.8
	Juice	76.4 ± 4.3	93.4 ± 6.1	87.9 ± 3.2
	Pomace	78.4 ± 6.7	88.5 ± 5.7	92.5 ± 4.7
	Apple products	**0.005 μg/g ***	**0.2 μg/g**	**2.0 μg/g**
ETU	Slices	80.5 ± 5.3	95.0 ± 5.6	96.3 ± 2.8
	Unpeeled sauce	82.4 ± 2.8	87.7 ± 2.9	99.8 ± 3.0
	Peeled sauce	79.9 ± 6.9	93.5 ± 5.5	96.7 ± 7.2
	Juice	75.7 ± 5.6	88.6 ± 7.1	98.1 ± 5.3
	Pomace	80.4 ± 5.2	85.3 ± 5.2	90.5 ± 8.2

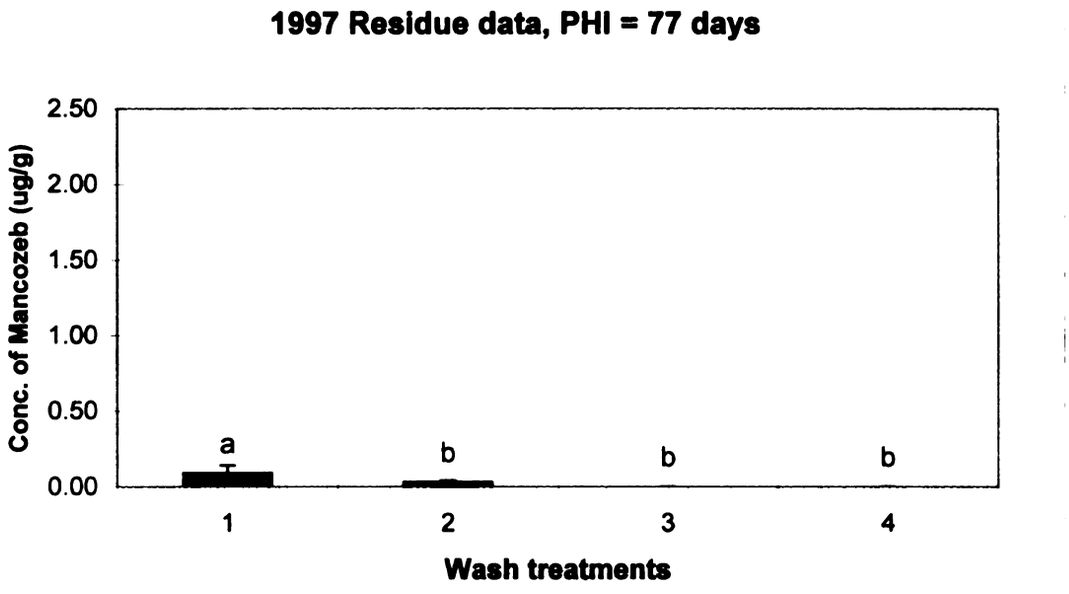
Note: 1. * Method detection limit (MDL) for mancozeb and ETU.
2. ** Values are the means of triplicate determinations.

C. Mancozeb Residue Study

(I) Comparison of Postharvest Wash Treatment on the Reduction of Mancozeb Residues

Control samples were subjected to the same postharvest treatment parameters except that they were not exposed to the mancozeb

during the growing seasons. The data illustrated in Figures 53–58, show the effects of the various wash treatments on reduction of mancozeb residues in/on apple and apple products. The total amount of residue on the unwashed apple was determined to be 1.99 ppm. The established tolerance level for mancozeb as published in the Code of Federal Regulation, USA (1996) is 3.0 ppm. There were no statistical differences between water wash and 50 ppm chlorine wash treatments (Figure 53). For 500 ppm chlorine and 3 ppm ozone treatments, no mancozeb residues were detected. Chlorine dioxide and HPAA were shown to be effective treatments compared to no–wash, water–wash or 50 ppm chlorine–wash; however, less effective than 500 ppm chlorine or ozone treatments. For slices, no significant differences were found among no–wash, water–wash and 50 ppm chlorine–wash treatments (Figure 54). This indicates that water wash only is insufficient in removing pesticide levels compared to no wash. In unpeeled sauce and peeled sauce, no mancozeb residues were detected in all wash–treated samples (Figures 55–56). In juice, there were no significant differences among no–wash, water–wash and 50 ppm chlorine–wash (Figure 57). High mancozeb residues were detected in 50 ppm chlorine–wash than water–wash. However, there was no significant difference between these two treatments. Five hundred ppm chlorine wash still showed a powerful effect among all treatments. In the case of pomace, relatively high levels



1. No wash
2. Water wash
3. 50 ppm chlorine
4. 500 ppm chlorine
5. 10 ppm chlorine dioxide
6. 3 ppm ozone
7. 50 ppm HPAA

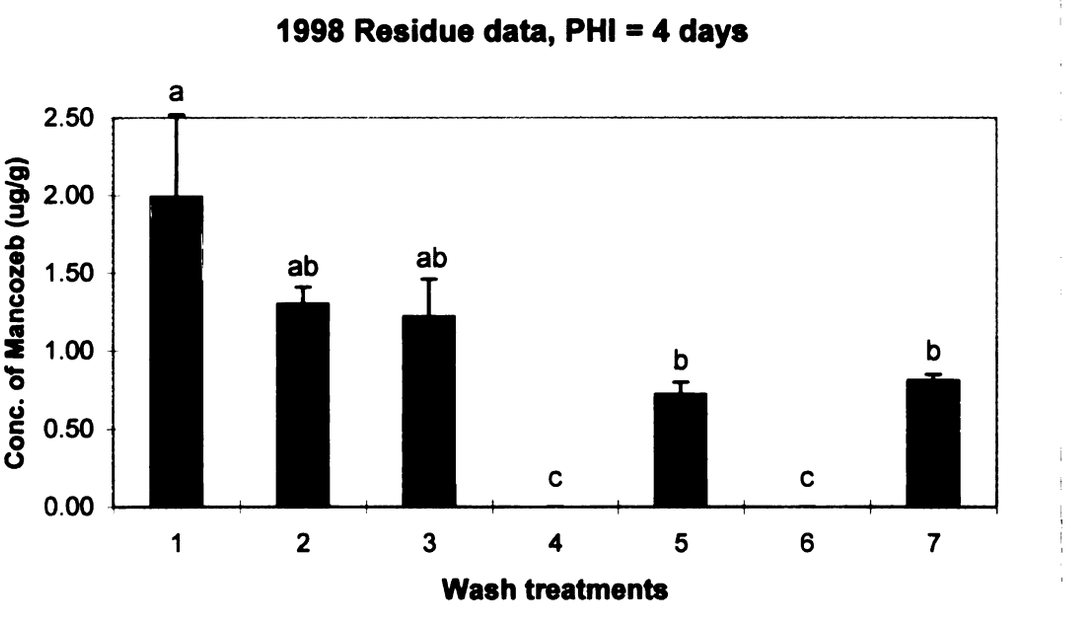
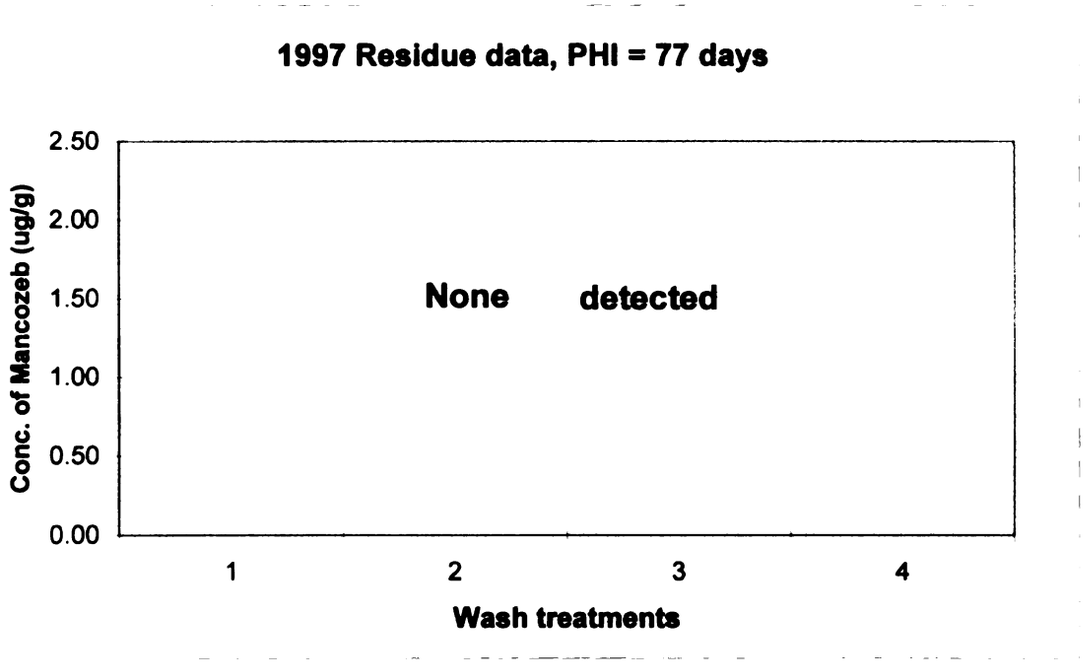


Figure 53. Concentration of Mancozeb residues in whole fruit after postharvest wash treatments.

* Values with same letters are not significantly different ($p<0.05$).



1. No wash
2. Water wash
3. 50 ppm chlorine
4. 500 ppm chlorine
5. 10 ppm chlorine dioxide
6. 3 ppm ozone
7. 50 ppm HPAA

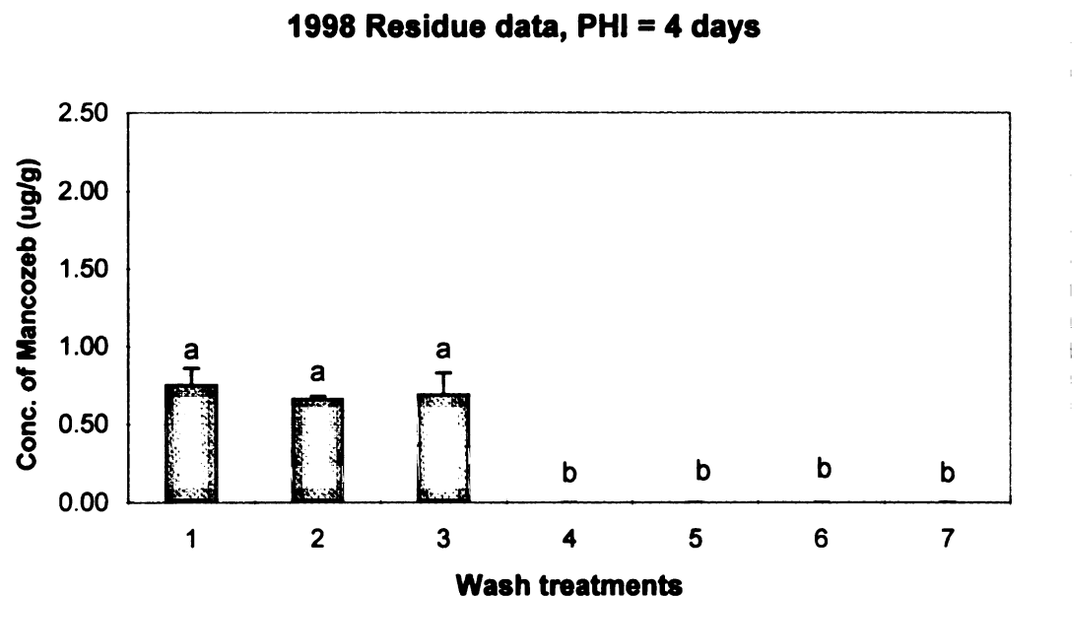
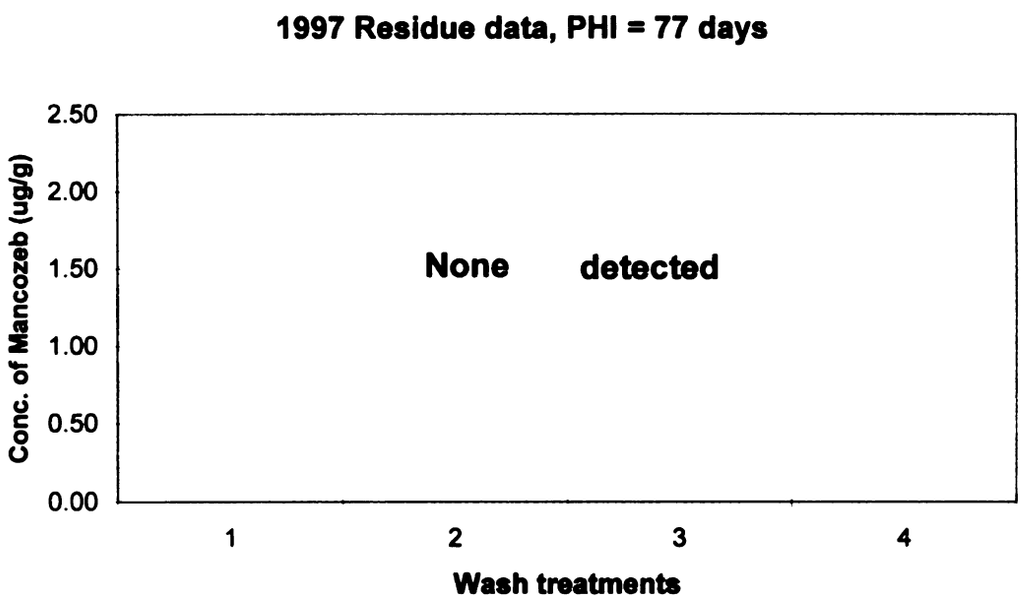


Figure 54. Concentration of Mancozeb residues in slices after postharvest wash treatments.

* Values with same letters are not significantly different ($p<0.05$).



1. No wash
2. Water wash
3. 50 ppm chlorine
4. 500 ppm chlorine
5. 10 ppm chlorine dioxide
6. 3 ppm ozone
7. 50 ppm HPAA

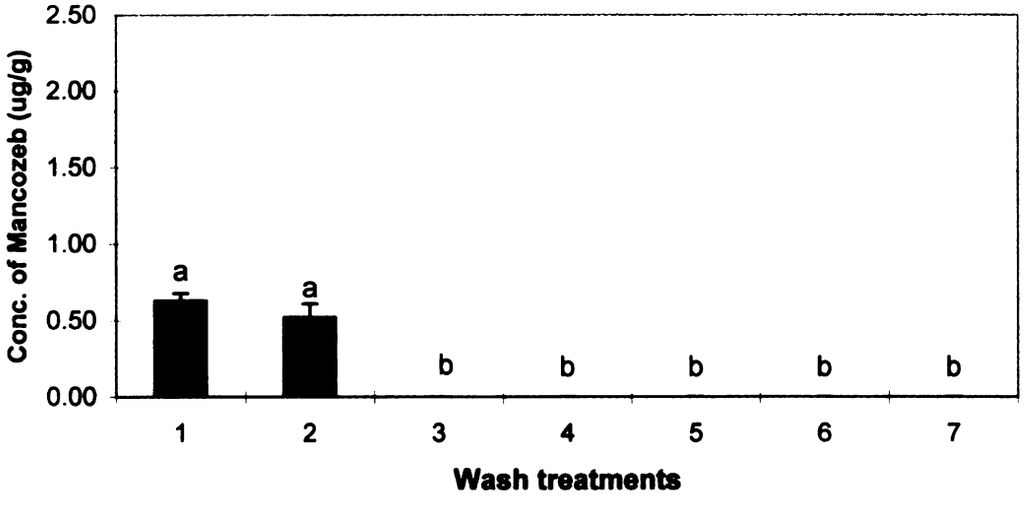
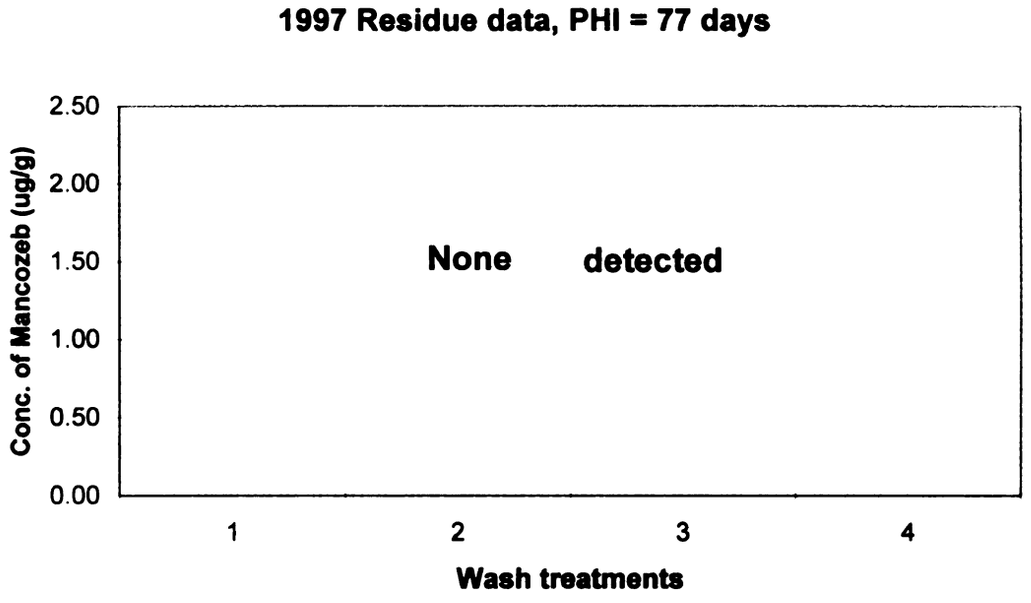


Figure 55. Concentration of Mancozeb residues in unpeeled sauce after postharvest treatments.

* Values with same letters are not significantly different ($p<0.05$).



1. No wash
2. Water wash
3. 50 ppm chlorine
4. 500 ppm chlorine
5. 10 ppm chlorine dioxide
6. 3 ppm ozone
7. 50 ppm HPAA

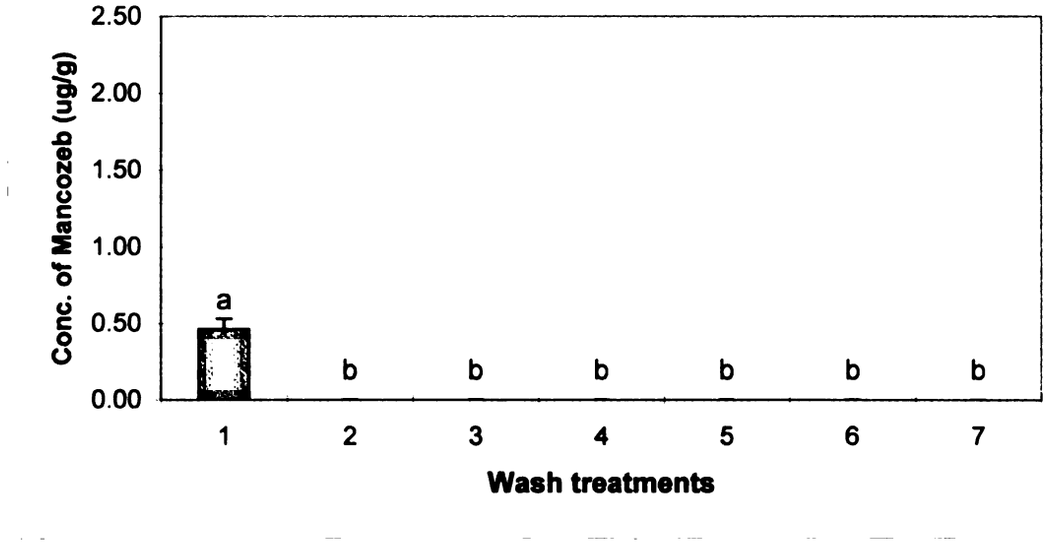
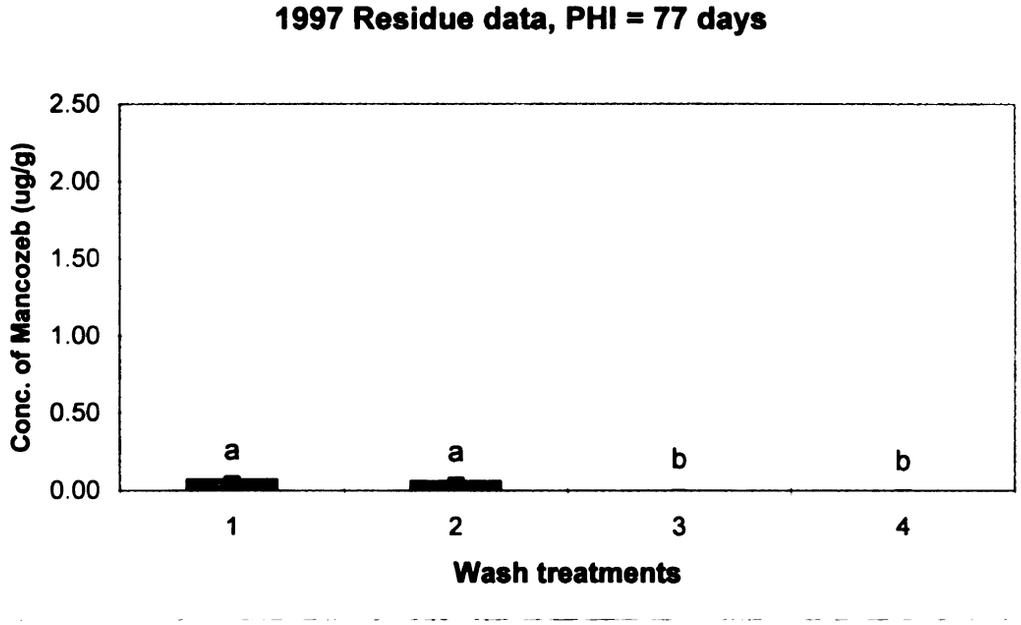


Figure 56. Concentration of Mancozeb residues in peeled sauce after postharvest treatments.

* Values with same letters are not significantly different ($p<0.05$).



1. No wash
2. Water wash
3. 50 ppm chlorine
4. 500 ppm chlorine
5. 10 ppm chlorine dioxide
6. 3 ppm ozone
7. 50 ppm HPAA

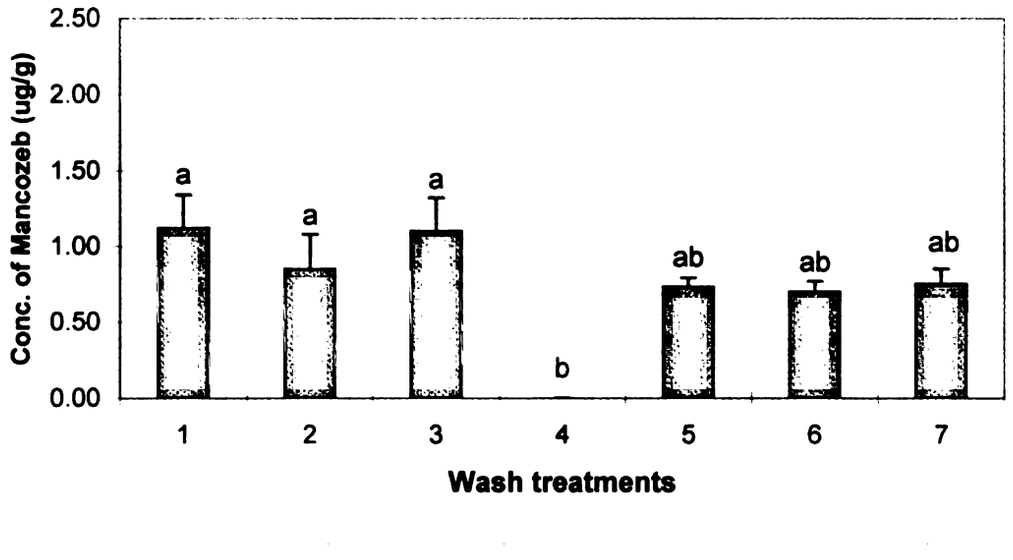


Figure 57. Concentration of Mancozeb residues in juice after postharvest wash treatments.

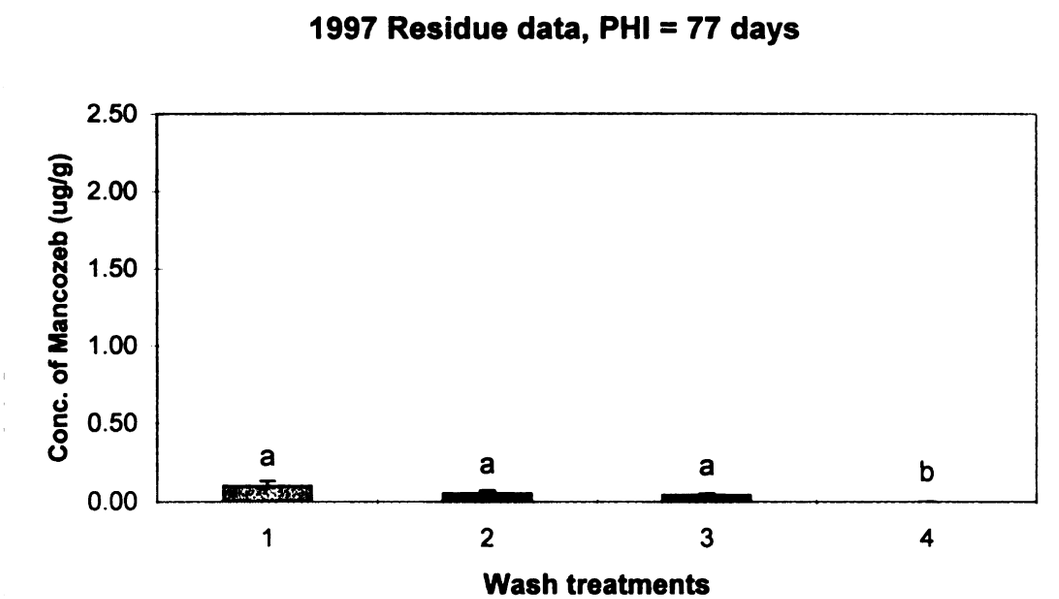
* Values with same letters are not significantly different ($p<0.05$).

of mancozeb were detected in all wash-treated samples (Figure 58). In the no-wash sample, the total concentration of mancozeb in pomace was 13.31 ppm which is much higher than the established tolerance level. Various wash treatments reduced mancozeb levels but were not effective compared to other products. In water-wash, 50 ppm chlorine and 10 ppm chlorine dioxide-wash, there were still high levels of mancozeb detected. Five hundred ppm chlorine and 3 ppm ozone treatments significantly ($p<0.05$) reduced mancozeb levels compared to other washes. Pomace may be used as feeds for livestock so much more concerns are needed.

(II) Comparison of Percent Reduction of Mancozeb Levels

The percent reduction in mancozeb residues in whole fruit, apple slices, unpeeled apple sauce, peeled apple sauce, juice and pomace are presented in Figures 59-64. To determine the percent residue reduction, each wash treated sample was compared to residues from the no wash treatment. The percent reduction in mancozeb was shown to decrease in relation to higher initial residue levels. The mancozeb residues in 1997 samples were lower than 1998 samples so, the overall percent reductions were high in 1997 studies.

Almost 50% of mancozeb residue was removed from the fruit with the water wash only in 1998 studies (Figure 59). In whole fruit,



1. No wash
2. Water wash
3. 50 ppm chlorine
4. 500 ppm chlorine
5. 10 ppm chlorine dioxide
6. 3 ppm ozone
7. 50 ppm HPAA

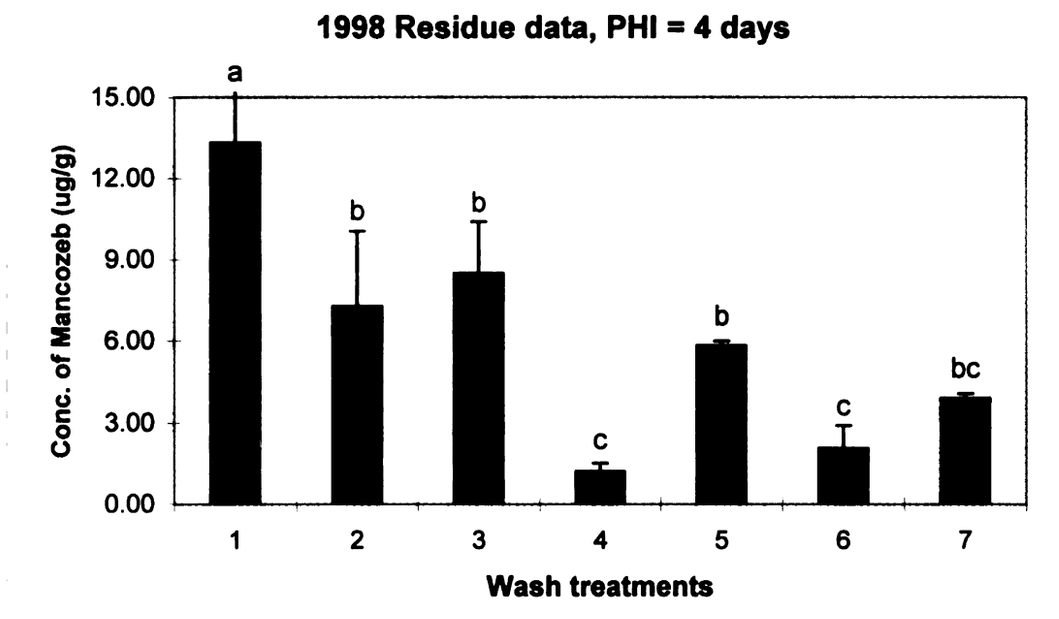
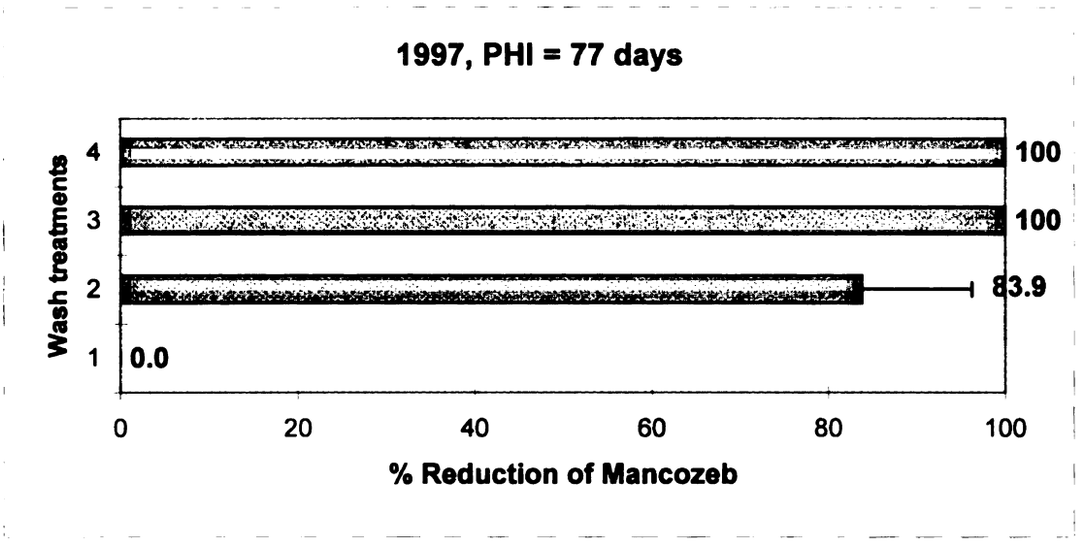


Figure 58. Concentration of Mancozeb residues in pomace after postharvest wash treatments.

* Values with same letters are not significantly different ($p<0.05$).



1. No wash
2. Water wash
3. 50 ppm chlorine
4. 500 ppm chlorine
5. 10 ppm chlorine dioxide
6. 3 ppm ozone
7. 50 ppm HPAA

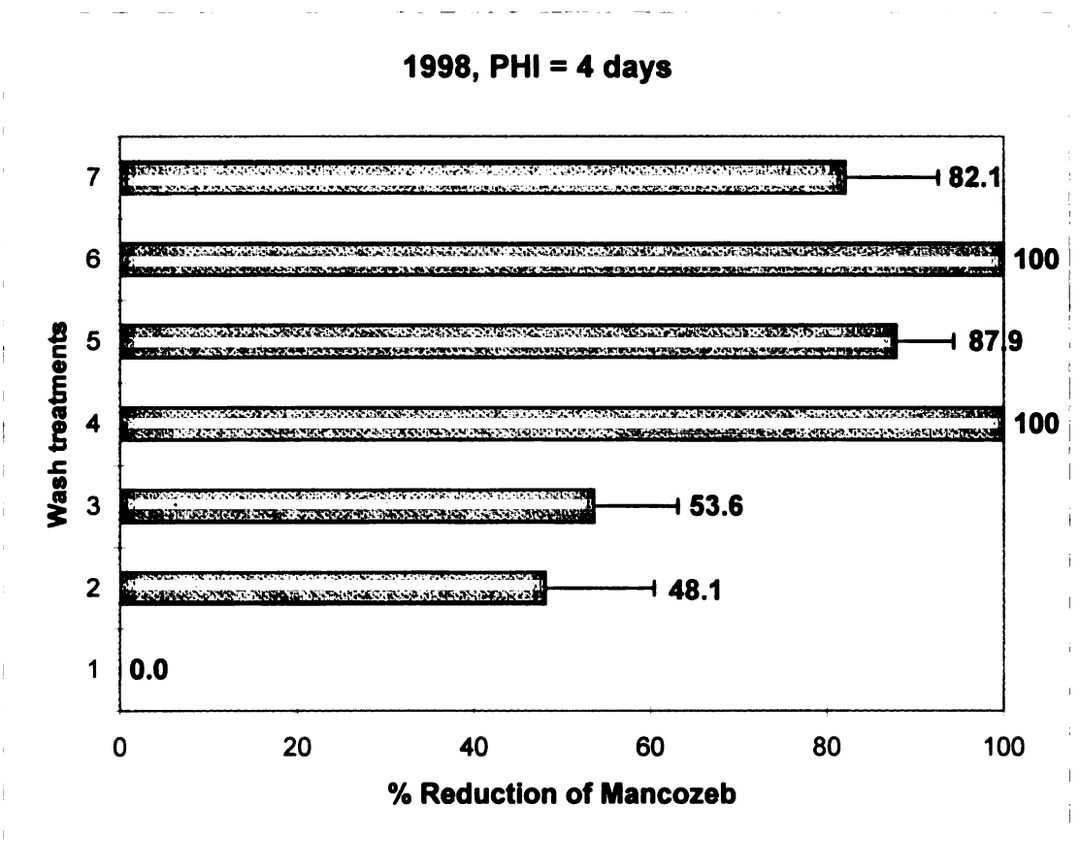
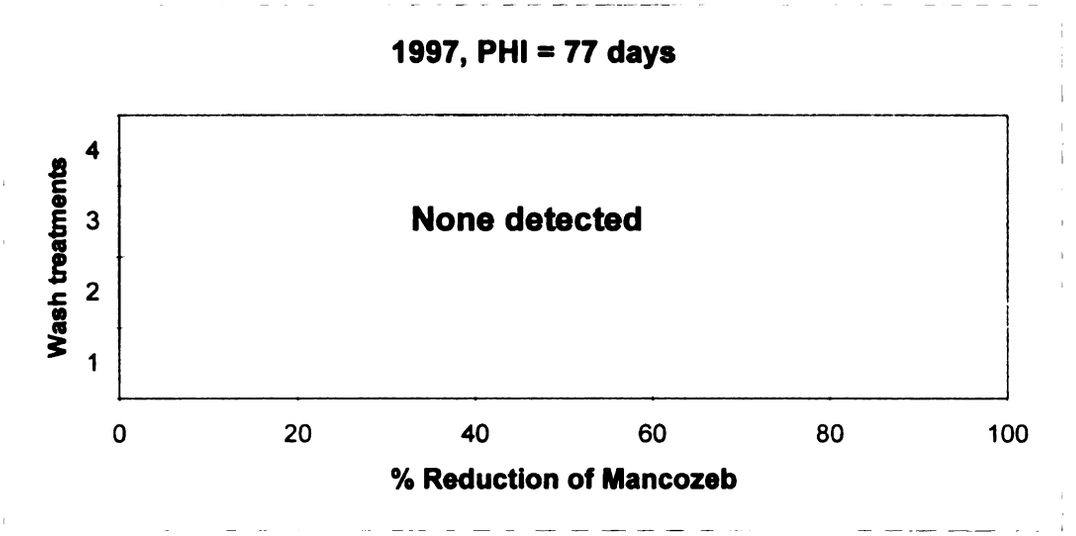


Figure 59. Percent reduction of Mancozeb residues in whole fruit after postharvest wash treatments.

chlorine wash at 50 and 500 ppm removed about 53% and 100% mancozeb residue, respectively in 1998 studies. Apples dipped in HPAA and ozonated water reduced residue levels by about 82% and 100%, respectively. While water alone reduced levels by 45%, the various other treatments reduced mancozeb by 50–100%. No statistical difference appeared between water–wash and 50 ppm chlorine–wash at the 5% level. Also, there was no significant ($p<0.05$) difference between HPAA– and chlorine dioxide–washes. The 500 ppm chlorine– and ozone–washes were the most effective treatments for every apple product. Apple slices and apple juice showed percent reduction of 45–100% and 47–100%, respectively (Figure 60). No mancozeb residues were detected in 500 ppm chlorine, 10 ppm chlorine dioxide, 3 ppm ozone, and 50 ppm HPAA washes. Unwashed apples that were processed into unpeeled and peeled sauce showed 70% and 100% reduction, respectively in residue level compared to no washed whole fruit (Figures 61–62). Apples from the various treatments that were subsequently processed into peeled and unpeeled sauces showed mancozeb residue reductions of 100%. Percent reduction in juice is shown in Figure 63. Water–wash and chlorine–wash at 50 ppm removed about 47% and 56% mancozeb residue, respectively in 1998 studies. In HPAA and ozonated water, mancozeb was reduced about 63% and 82%, respectively. Chlorine at 500 ppm gave the best effect on the reduction of mancozeb residue. Percent reduction of



1. No wash
2. Water wash
3. 50 ppm chlorine
4. 500 ppm chlorine
5. 10 ppm chlorine dioxide
6. 3 ppm ozone
7. 50 ppm HPAA

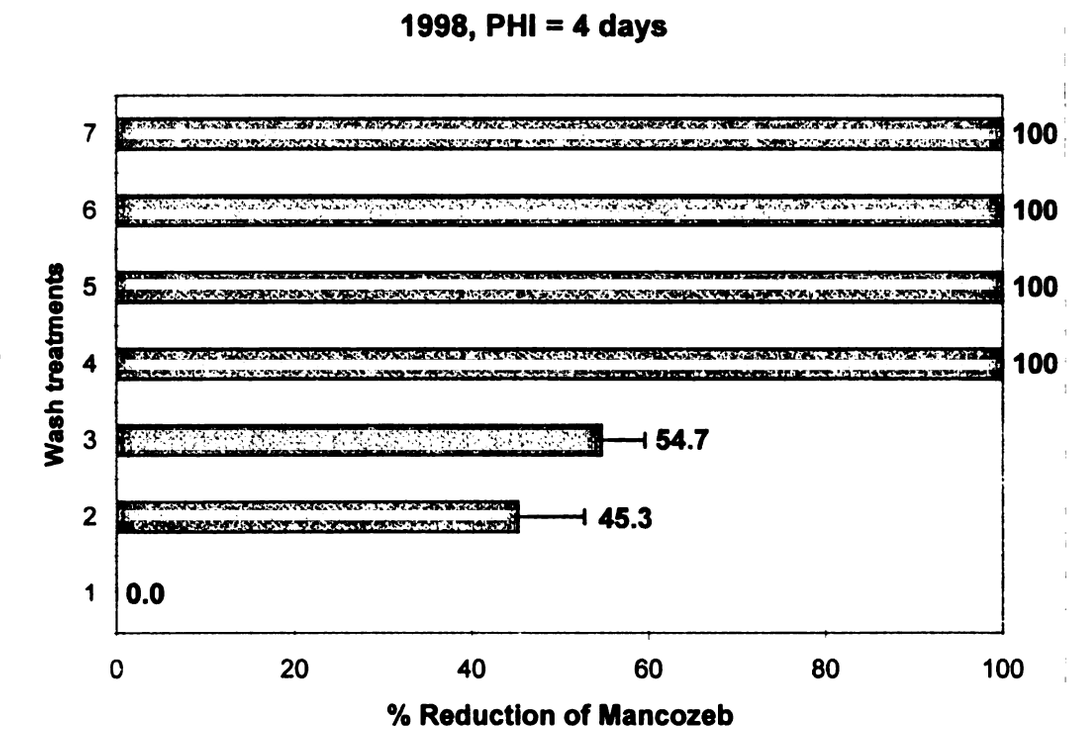
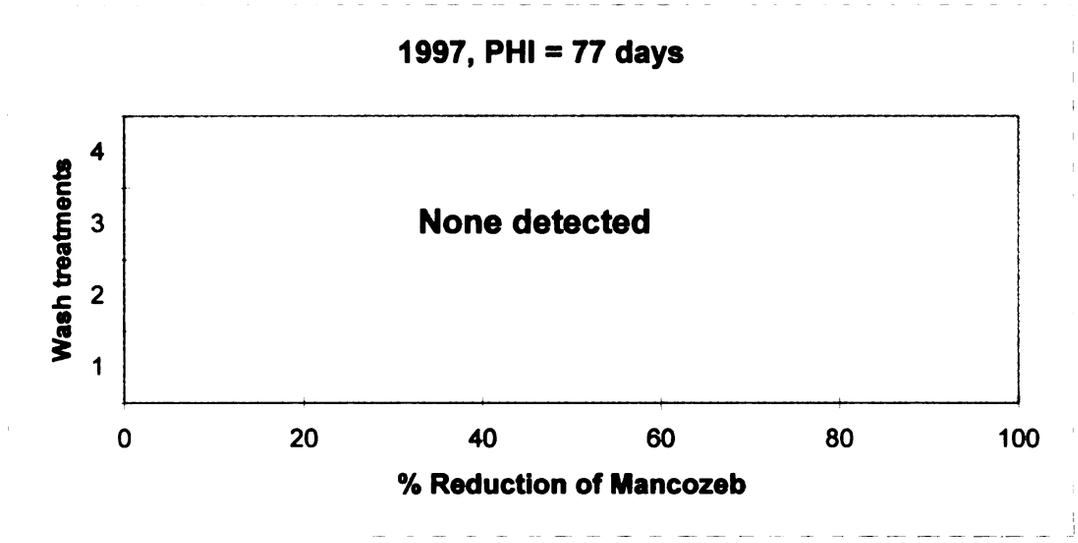


Figure 60. Percent reduction of Mancozeb residues in slices after Postharvest Wash Treatments.



1. No wash
2. Water wash
3. 50 ppm chlorine
4. 500 ppm chlorine
5. 10 ppm chlorine dioxide
6. 3 ppm ozone
7. 50 ppm HPAA

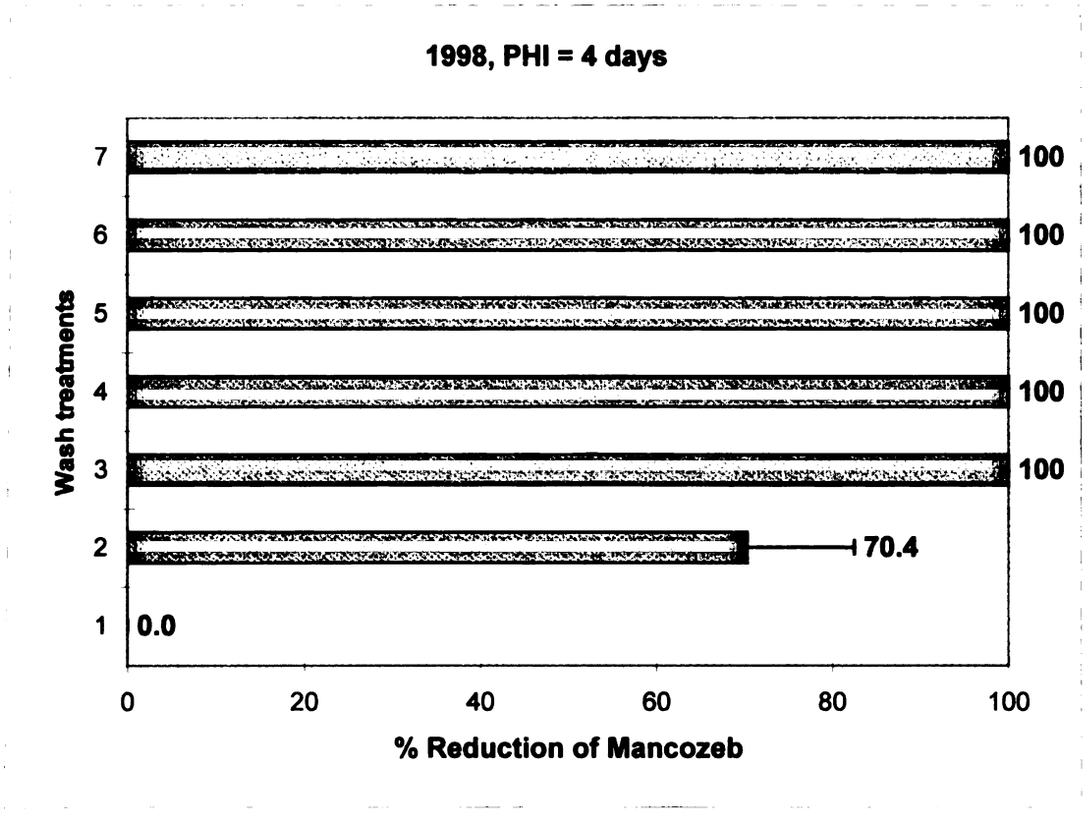


Figure 61. Percent reduction of Mancozeb residues in unpeeled sauce after postharvest wash treatments.

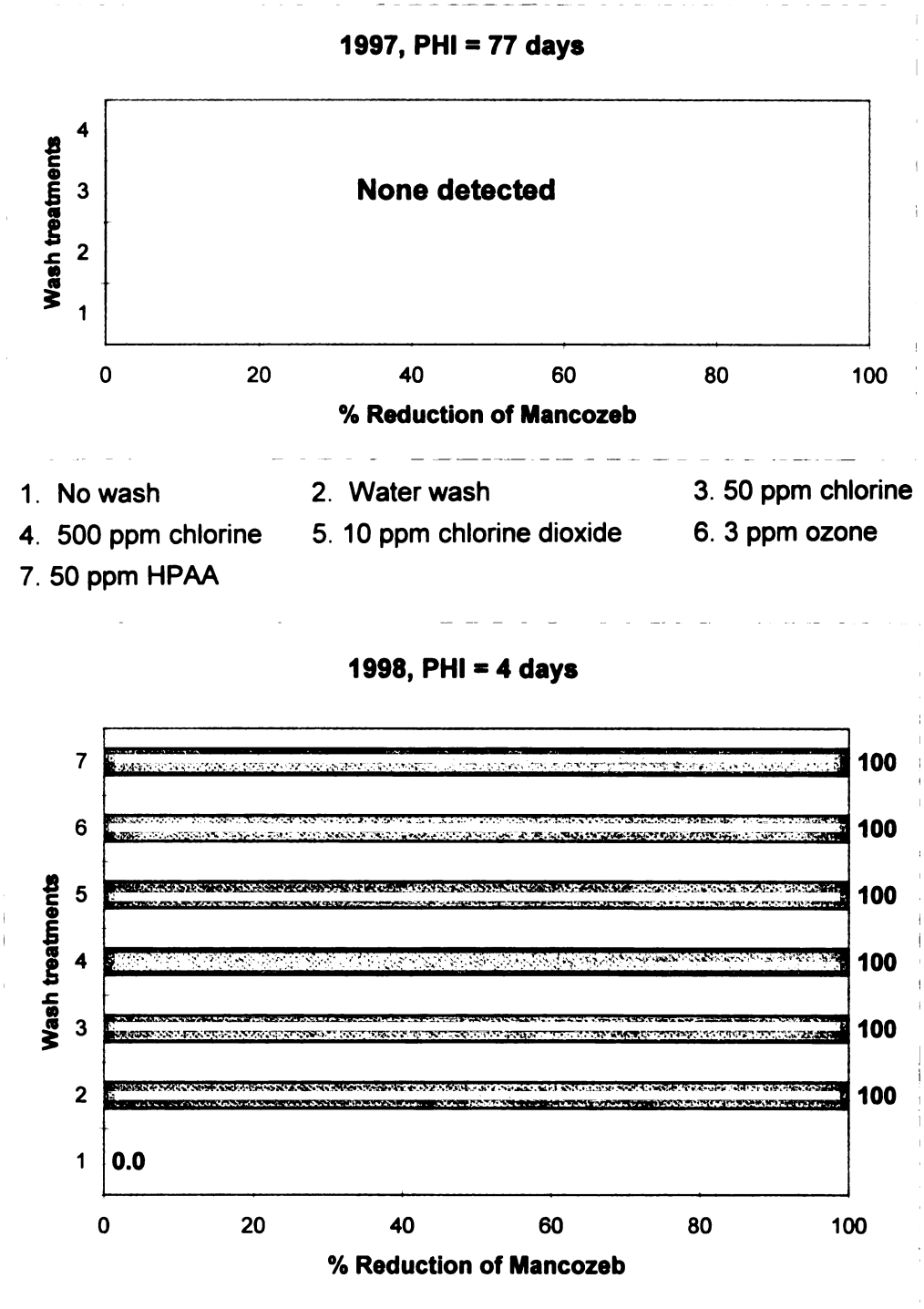
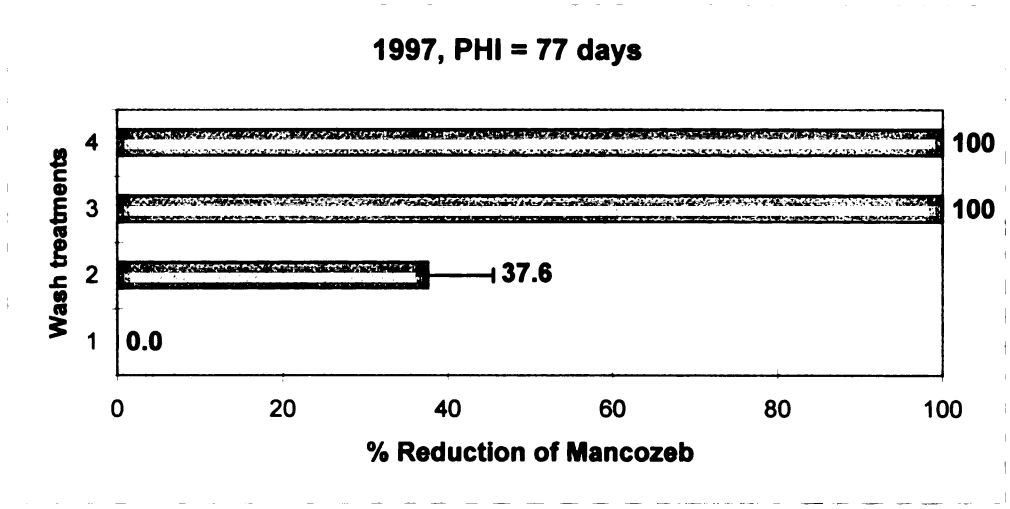


Figure 62. Percent reduction of Mancozeb residues in peeled sauce after postharvest wash treatments.



1. No wash
2. Water wash
3. 50 ppm chlorine
4. 500 ppm chlorine
5. 10 ppm chlorine dioxide
6. 3 ppm ozone
7. 50 ppm HPAA

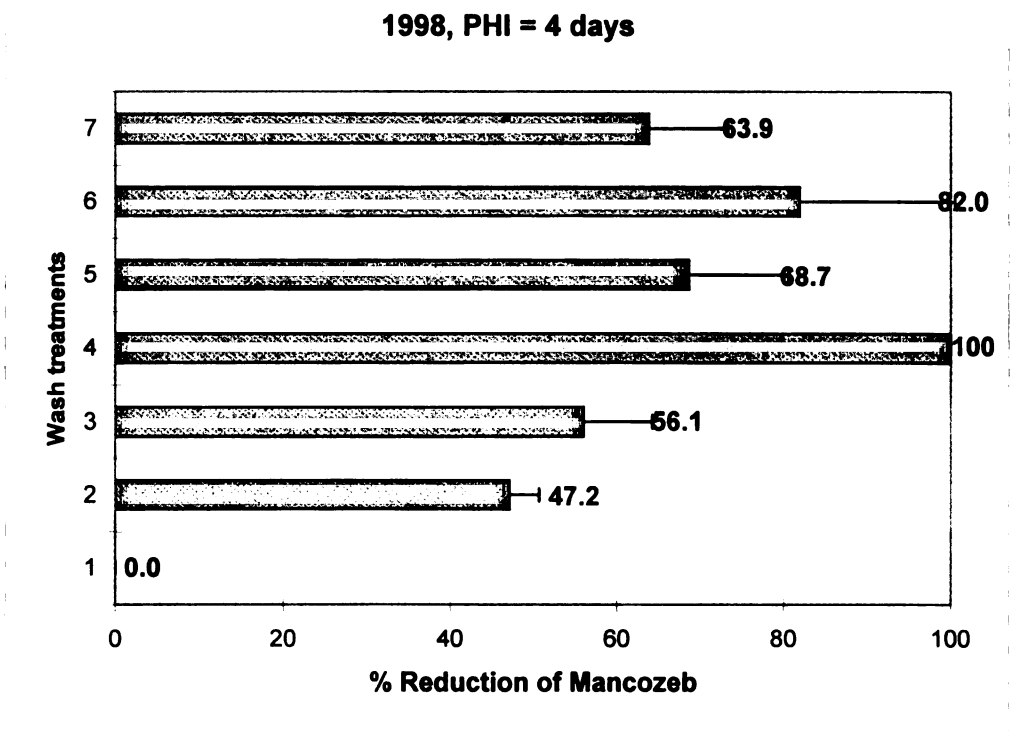
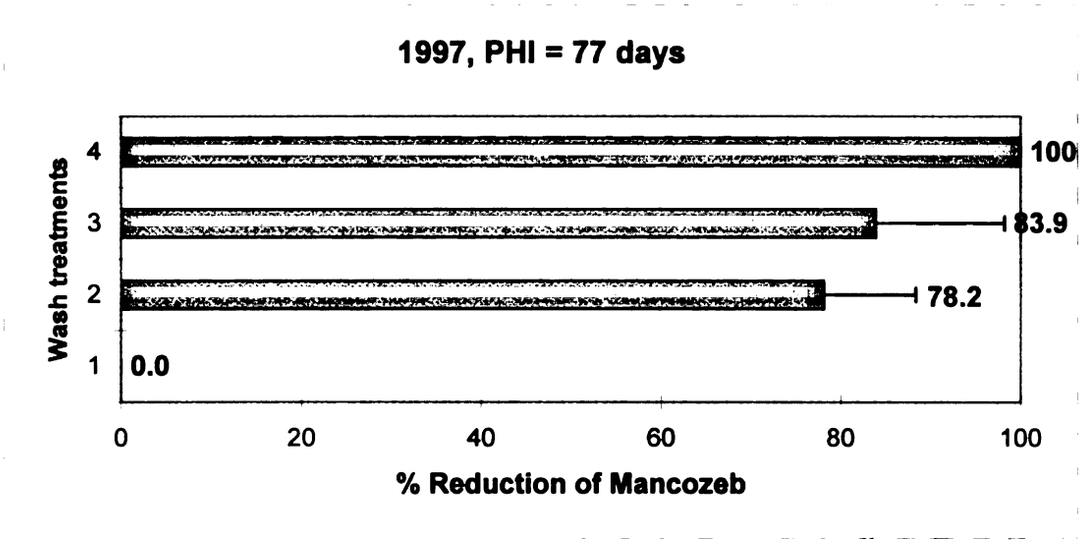


Figure 63. Percent reduction of Mancozeb residues in juice after Postharvest Wash Treatments.

mancozeb in pomace showed patterns similar to other products; however, the rate of reduction was lower (Figure 64). Water–wash seemed to be more effective than chlorine wash at 50 ppm but there was no statistically significant ($p<0.05$) difference. The patterns of overall percent reduction was found to be consistent in all treatments.

(III) Comparison of Mancozeb Residue Levels between Products

The differences observed in the residue levels between products is a direct result of the different processing methods used. In no–wash and water–wash treatments, the differences in residues observed between the two apple sauces is a result of peeling. When removing the peel we also remove any residue which may bound on the surface of skin. Processing into peeled apple sauce completely eliminated the mancozeb in all wash–treatments. Mancozeb is a contact fungicide which resides on the surface of the fruits so it would be assumed that removing the peel would give a high reduction of mancozeb residues. Processing the apples into slices, unpeeled sauce or peeled sauce significantly reduced all residues in all the treatments. When apples were pressed for juice the residues in the resulting pomace were concentrated and the percent reduction of mancozeb in pomace by various washes was less than in the other products. This was not unexpected, since a given weight of pomace represents 4 to 7 times its weight of raw product before



1. No wash
2. Water wash
3. 50 ppm chlorine
4. 500 ppm chlorine
5. 10 ppm chlorine dioxide
6. 3 ppm ozone
7. 50 ppm HPAA

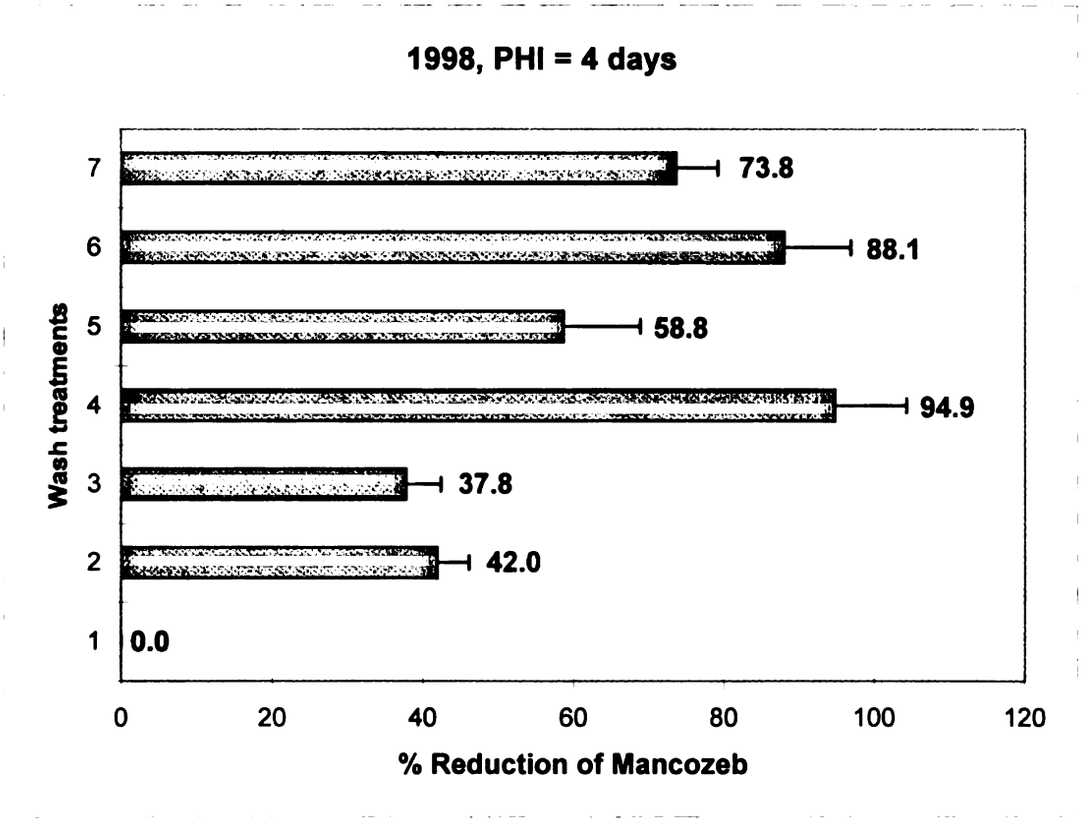


Figure 64. Percent reduction of Mancozeb residues in pomace after Postharvest Wash Treatments.

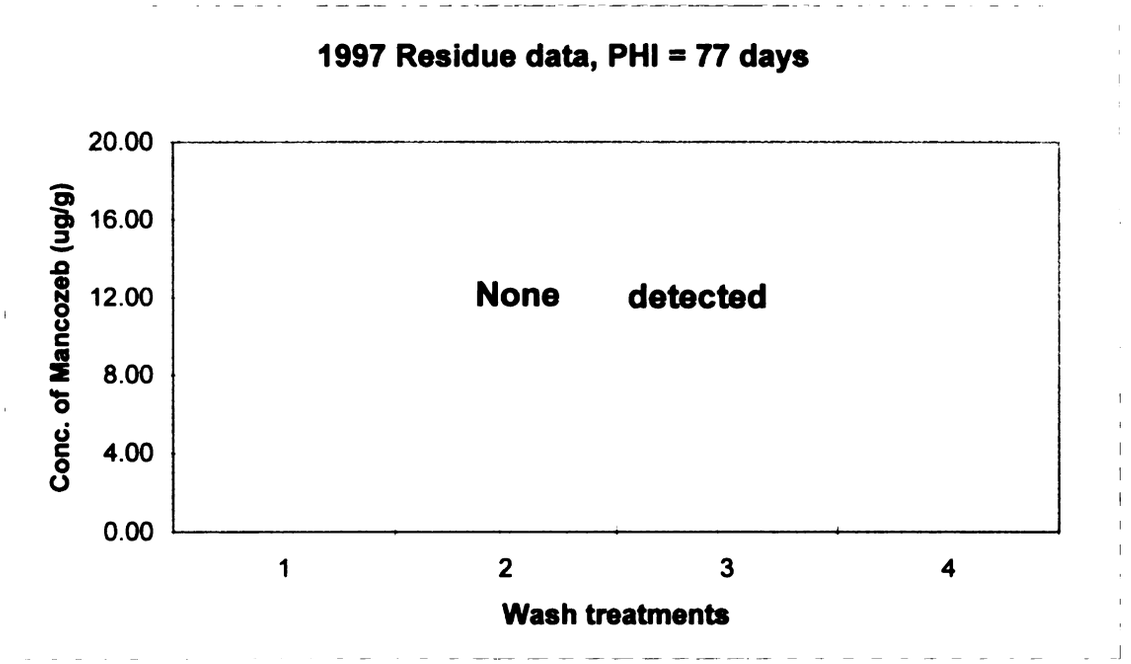
crushing and processing. Even so, the reductions by 500 ppm chlorine and ozone were still significant. Apple pomace is the principal solid waste generated in the apple processing industry. This is converted to various marketable by-products such as animal feed, pectin or natural fiber extract (Downing, 1989).

Overall, high reductions in mancozeb residues were observed in all products from the combination of postharvest wash treatments and processing. These findings are in agreement with previous studies by Ong *et al.*(1996) and Siler (1998) on the ability of washing and processing to significantly reduce pesticide residues in apples as well as other fruits and vegetables. In the study by El–Hadidi (1993), processing of apples into apple products such as apple slices, sauce and juice were significantly effective in reducing the pesticide residue levels to non-detectable amounts.

D. ETU Residue Study

(I) Comparison of Postharvest Wash Treatment on the Reduction of ETU Residues

The data presented Figures 65–70, show the effects of the various wash treatments on reduction of ETU residues in both 1997 and 1998 studies. The total amount of residue on the unwashed apples was determined to be 0.02 ppm. ETU is a possible human carcinogen so its



1. No wash
2. Water wash
3. 50 ppm chlorine
4. 500 ppm chlorine
5. 10 ppm chlorine dioxide
6. 3 ppm ozone
7. 50 ppm HPAA

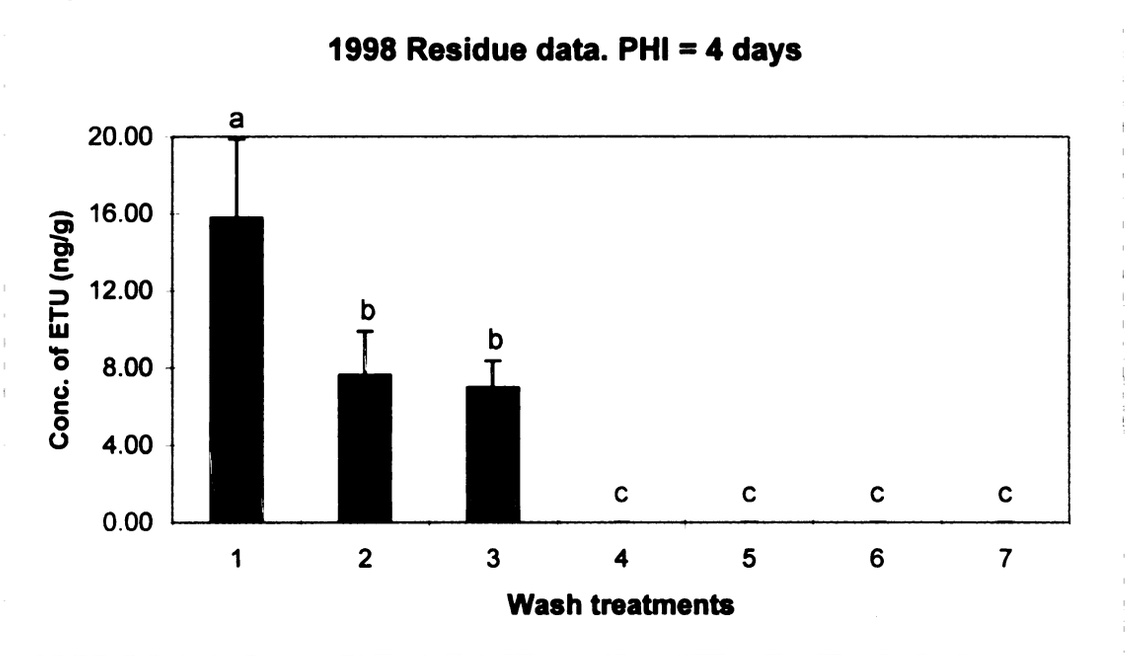
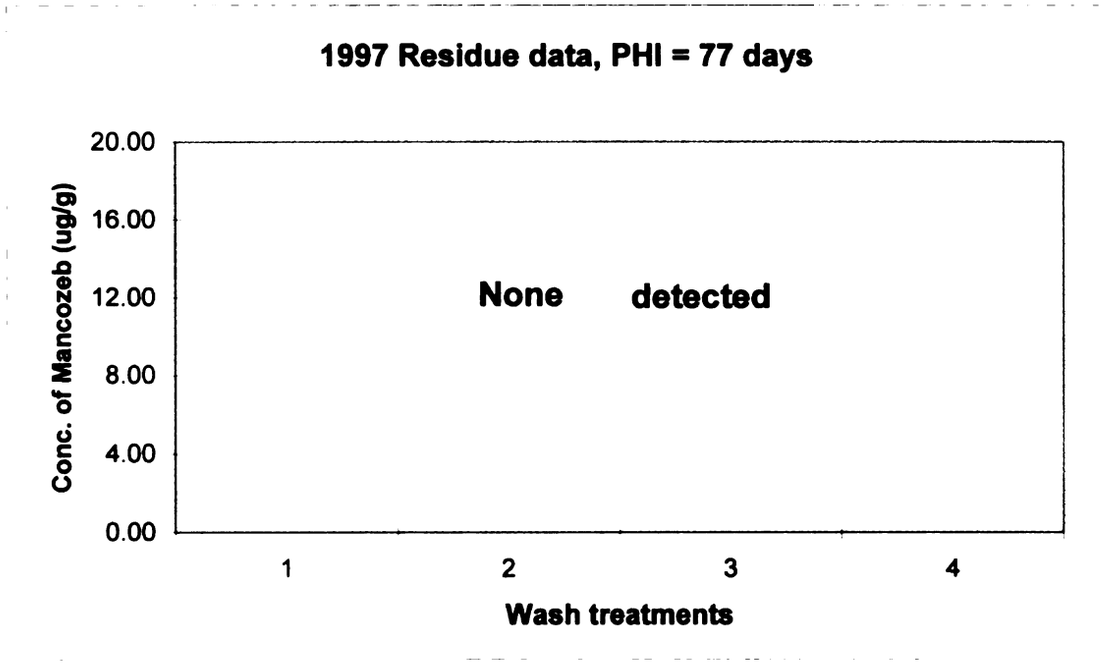


Figure 65. Concentration of ETU residues in whole fruit after postharvest wash treatments.

* Values with same letters are not significantly different ($p<0.05$).



1. No wash
2. Water wash
3. 50 ppm chlorine
4. 500 ppm chlorine
5. 10 ppm chlorine dioxide
6. 3 ppm ozone
7. 50 ppm HPAA

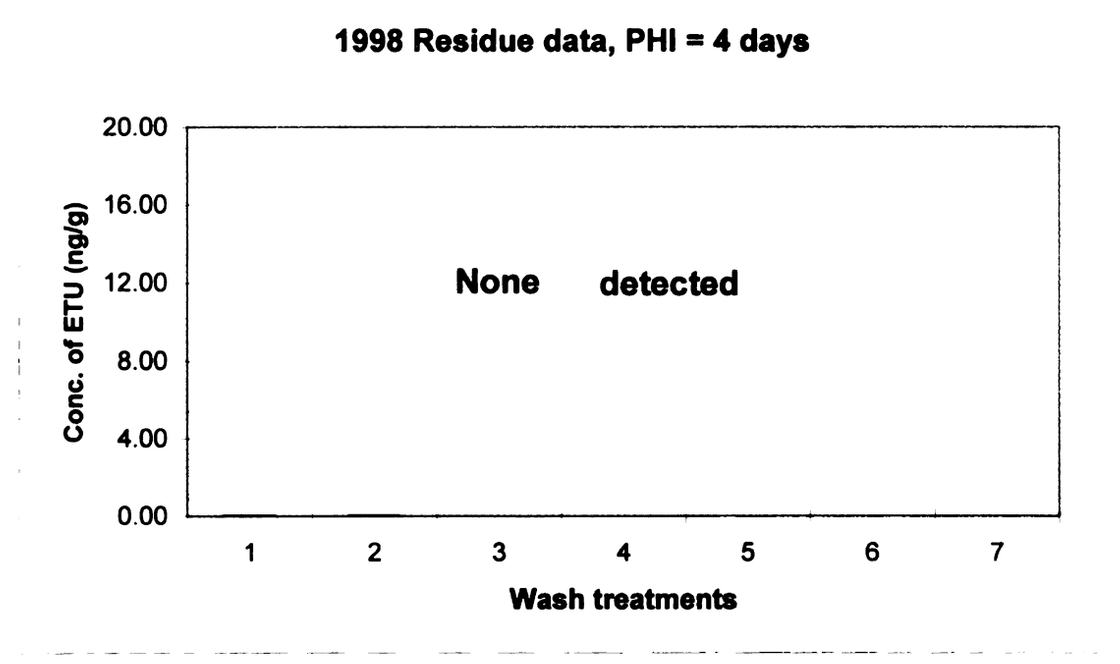
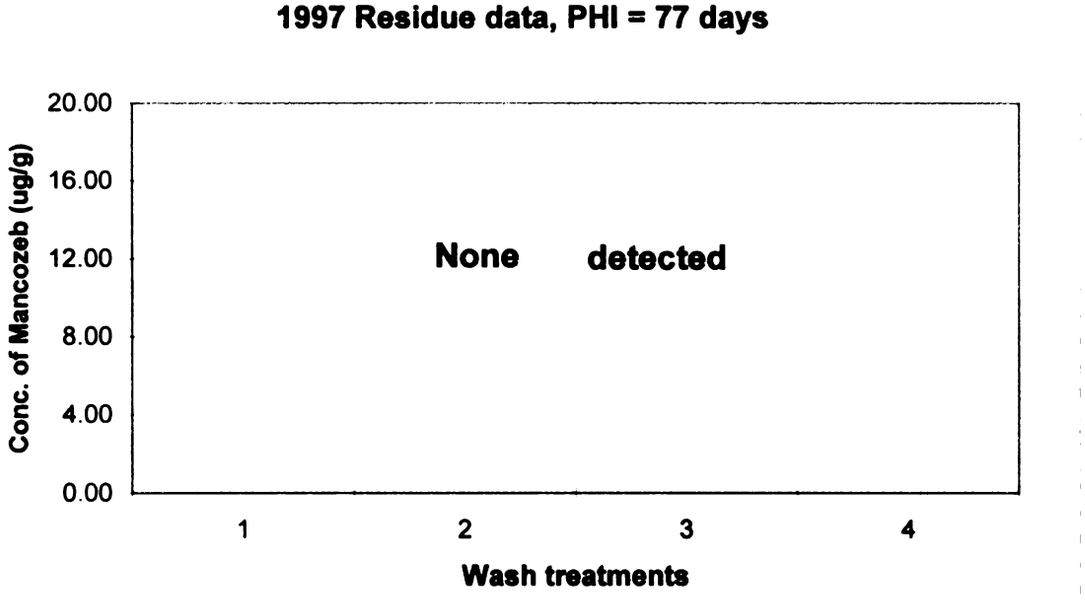


Figure 66. Concentration of ETU residues in slices after postharvest wash treatments.

* Values with same letters are not significantly different ($p<0.05$).



1. No wash
2. Water wash
3. 50 ppm chlorine
4. 500 ppm chlorine
5. 10 ppm chlorine dioxide
6. 3 ppm ozone
7. 50 ppm HPAA

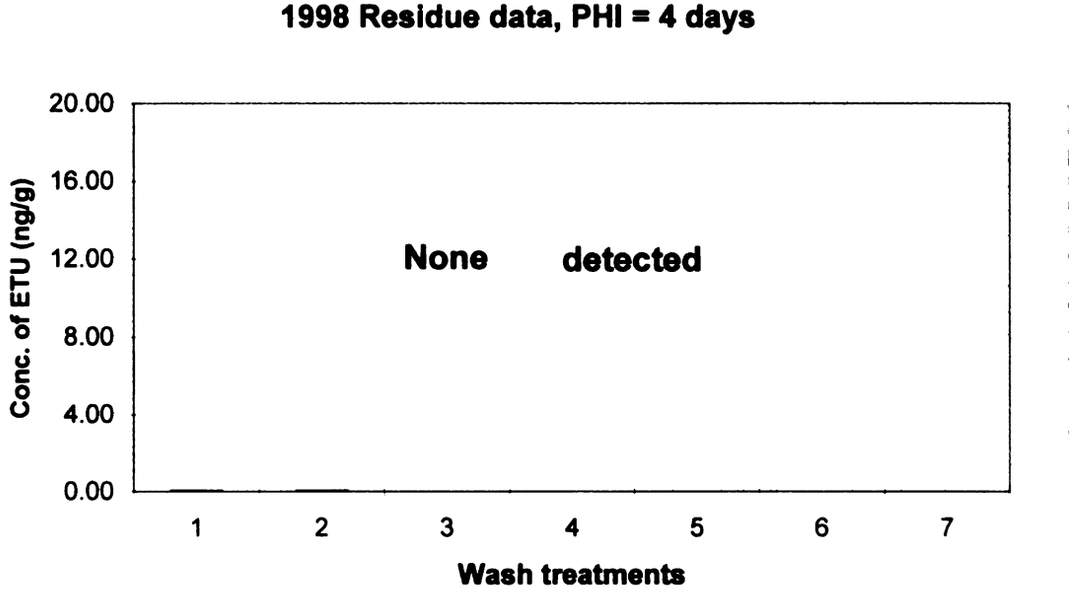
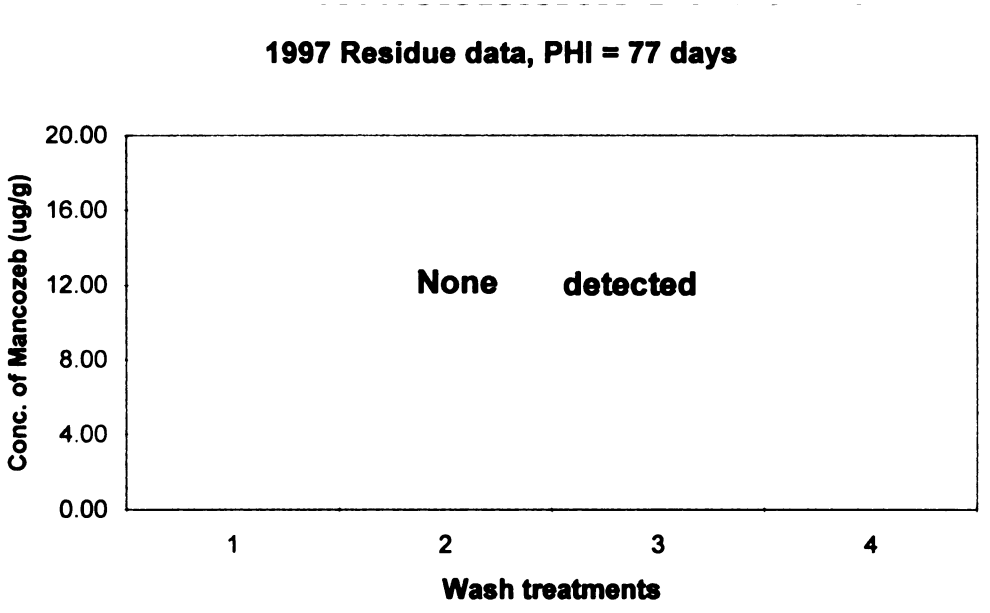


Figure 67. Concentration of ETU residues in unpeeled sauce after postharvest wash treatments.

* Values with same letters are not significantly different ($p<0.05$).



1. No wash
2. Water wash
3. 50 ppm chlorine
4. 500 ppm chlorine
5. 10 ppm chlorine dioxide
6. 3 ppm ozone
7. 50 ppm HPAA

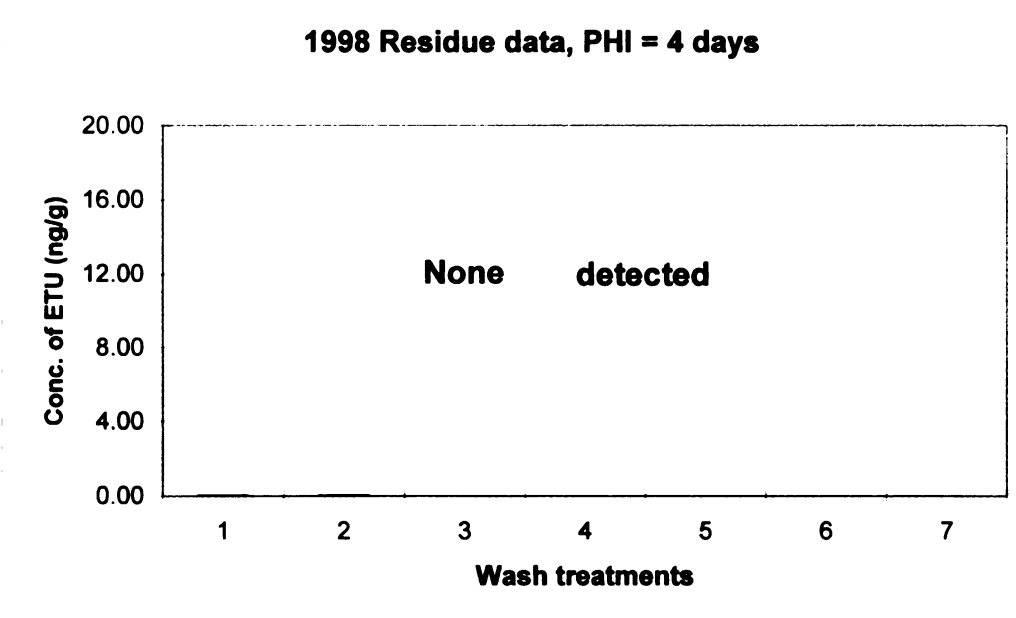
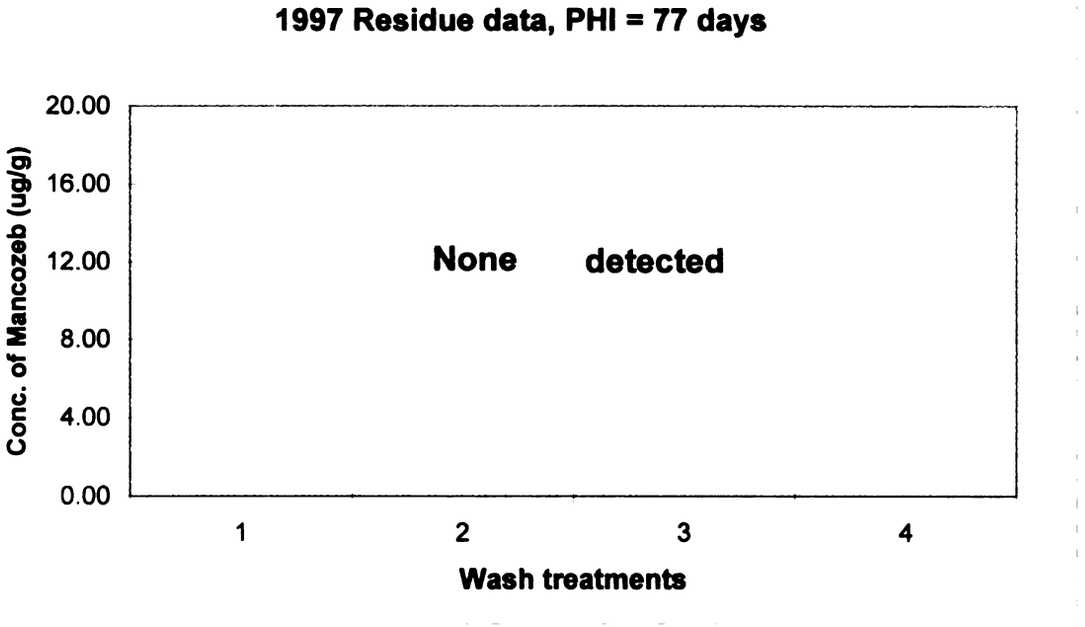


Figure 68. Concentration of ETU residues in peeled sauce after postharvest wash treatments.

* Values with same letters are not significantly different ($p<0.05$).



1. No wash
2. Water wash
3. 50 ppm chlorine
4. 500 ppm chlorine
5. 10 ppm chlorine dioxide
6. 3 ppm ozone
7. 50 ppm HPAA

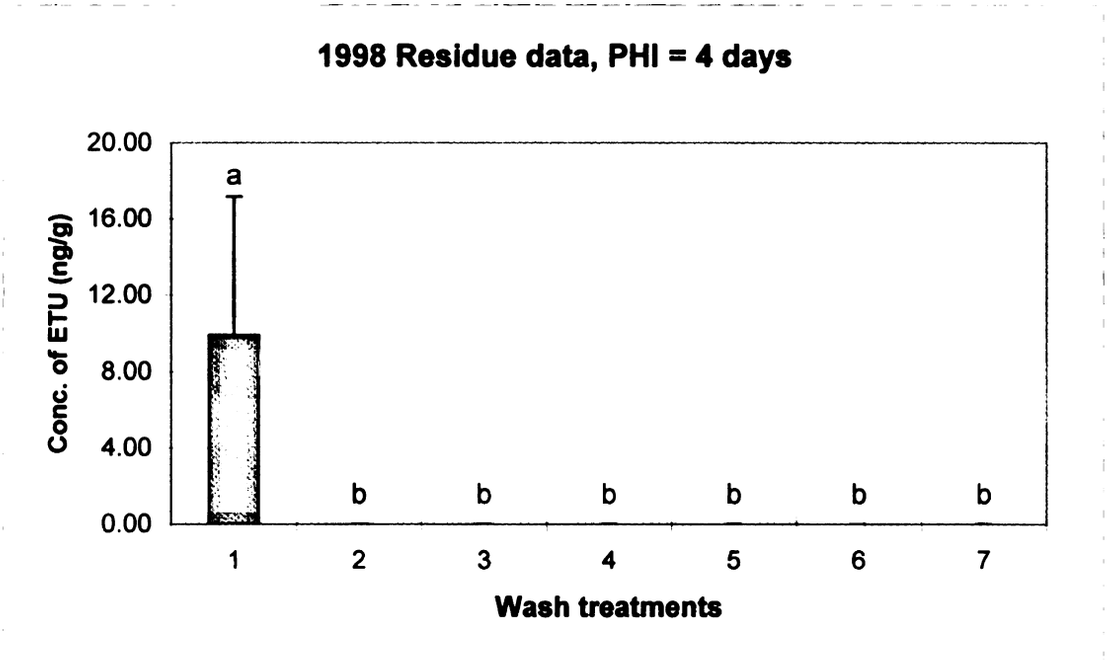
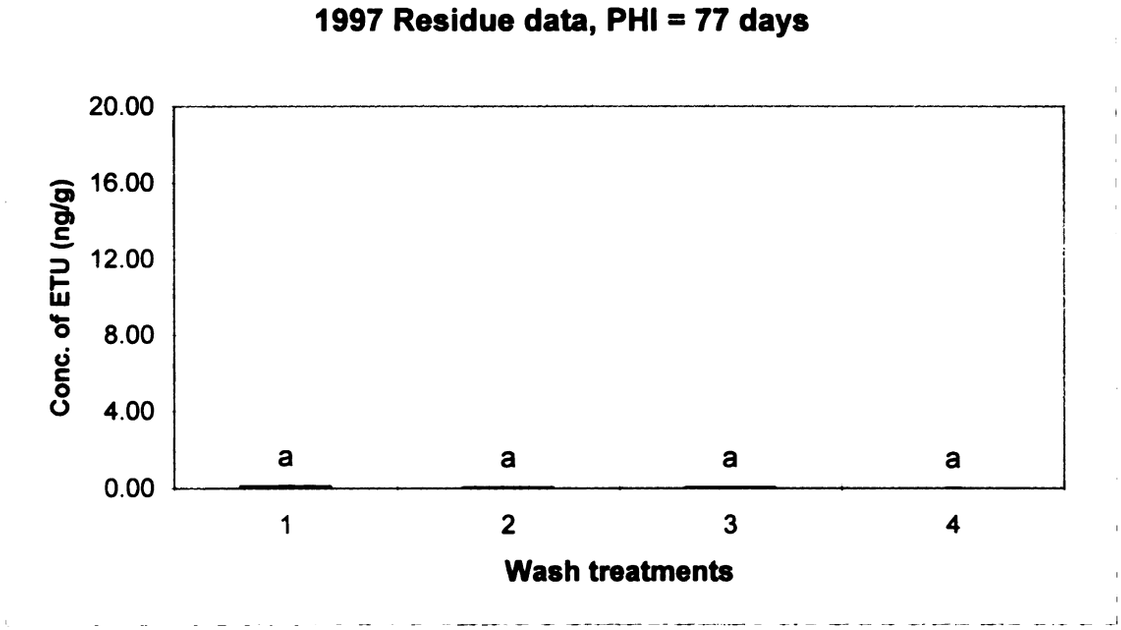


Figure 69. Concentration of ETU residues in juice after postharvest wash treatments.

* Values with same letters are not significantly different ($p<0.05$).



1. No wash
2. Water wash
3. 50 ppm chlorine
4. 500 ppm chlorine
5. 10 ppm chlorine dioxide
6. 3 ppm ozone
7. 50 ppm HPAA

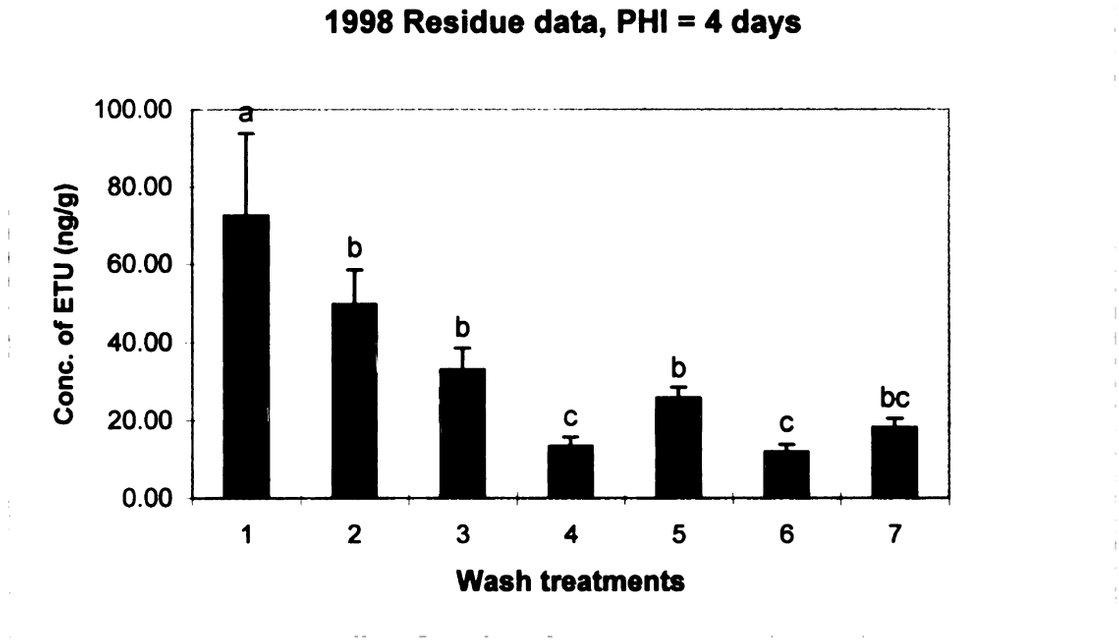


Figure 70. Concentration of ETU residues in pomace after postharvest wash treatments.

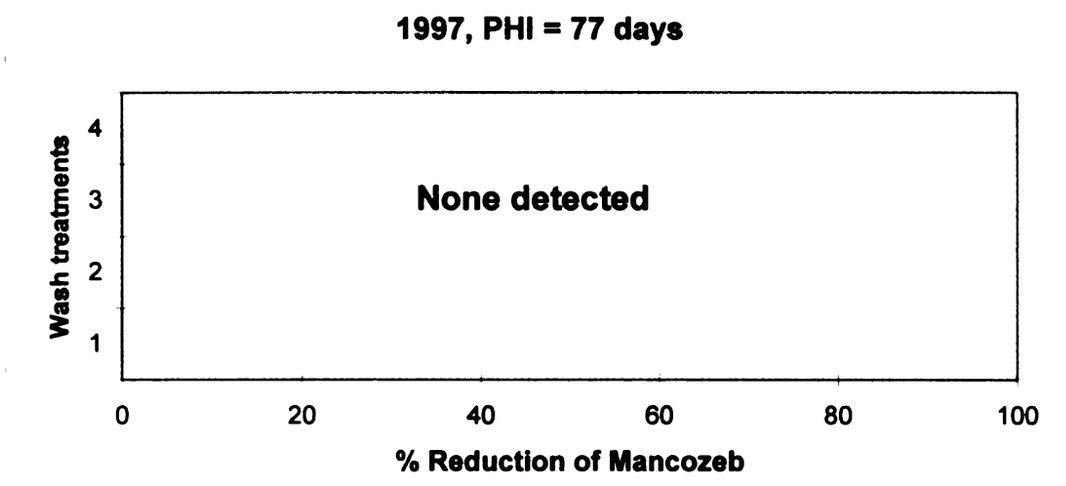
* Values with same letters are not significantly different ($p<0.05$).

presence is undesirable. In Figure 65, 500 ppm chlorine, chlorine dioxide ozone and HPAA treatments reduced ETU levels to non-detection limits in whole fruit. In slices, unpeeled sauce and peeled sauce, no ETU was found (Figures 66–68). In pomace, relatively high levels of ETU were detected in all wash treated samples (Figure 70). For the no–wash sample, the total concentration of ETU was 0.07 ppm. Various wash treatments reduced ETU concentration. For water–wash, 50 ppm chlorine and 10 ppm chlorine dioxide wash, high levels of ETU were detected. 500 ppm chlorine and 3 ppm ozone treatments significantly ($p<0.05$) reduced ETU levels compared to other washes.

(II) Comparison of Percent Reduction of ETU Levels

The percent reduction of ETU residues in whole fruit, apple slices, unpeeled apple sauce, peeled apple sauce, juice and pomace are presented in Figures 71–76. To calculate the percent ETU residue reduction, each wash–treated samples were compared to residues in each of the no wash treatments. The ETU residues in 1997 all samples and unpeeled and peeled sauce in 1998 samples were below the detection limit in all wash treatments. So these samples were excluded in the determination of percent reduction.

Almost 38% of ETU residue was removed from the fruit with the water wash only in 1998 studies (Figure 71). In whole fruit, chlorine



1. No wash
2. Water wash
3. 50 ppm chlorine
4. 500 ppm chlorine
5. 10 ppm chlorine dioxide
6. 3 ppm ozone
7. 50 ppm HPAA

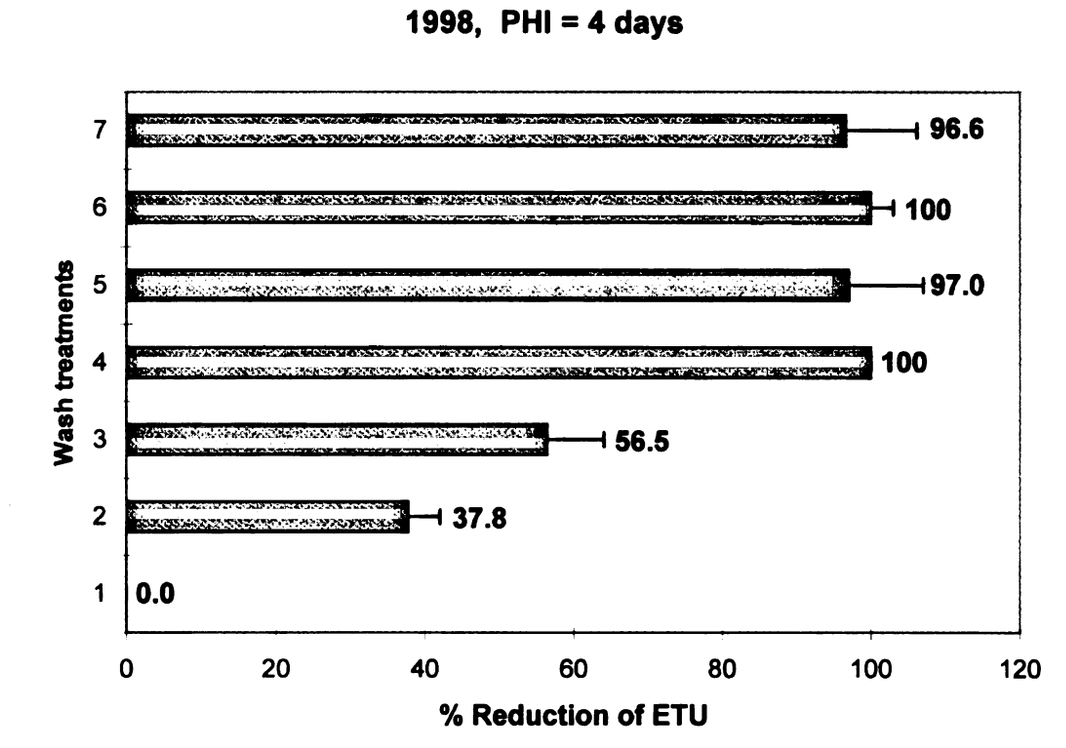


Figure 71. Percent reduction of ETU residues in whole fruit after Postharvest Wash Treatments.

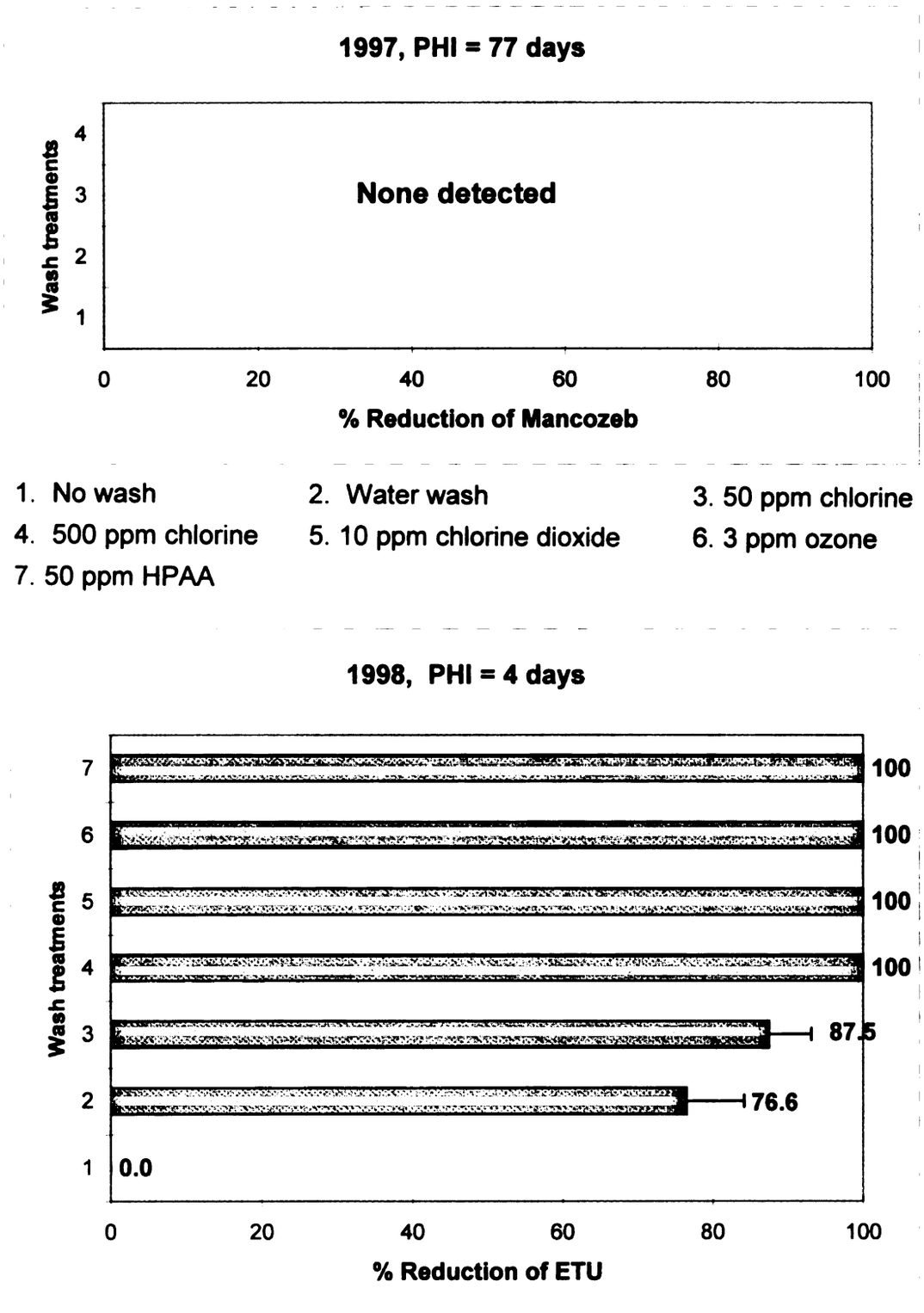


Figure 72. Percent reduction of ETU residues in slices after postharvest wash treatments.

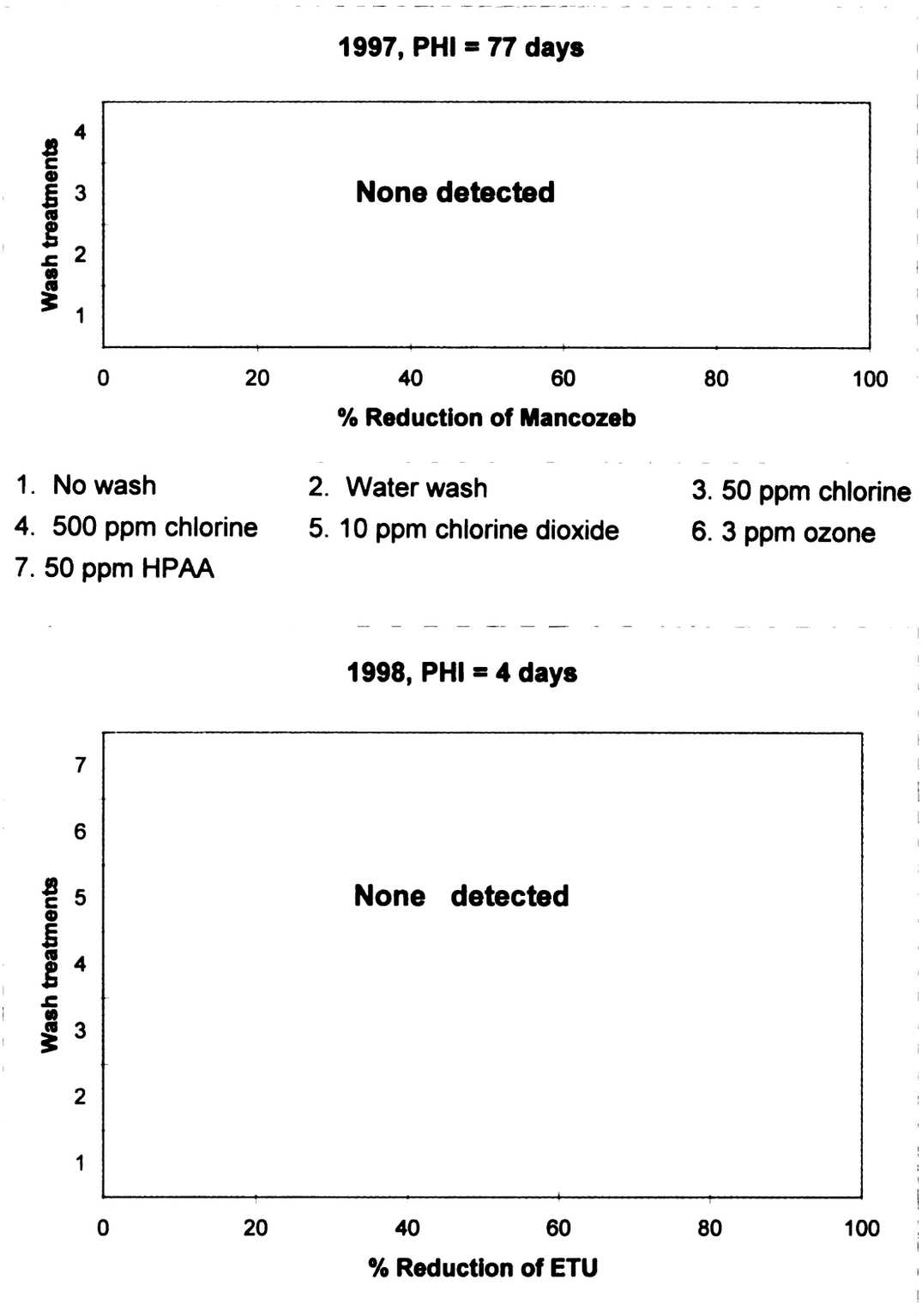
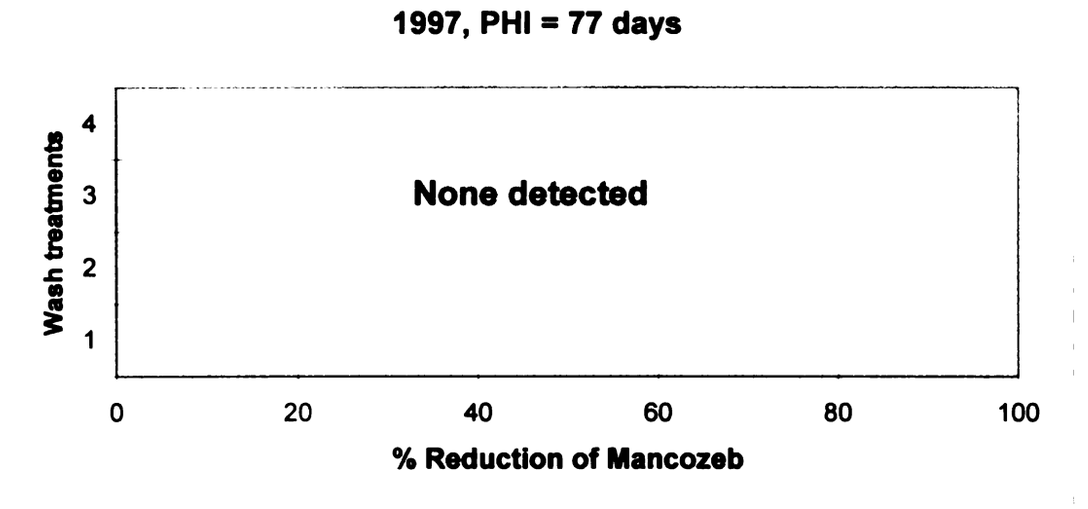


Figure 73. Percent reduction of ETU residues in unpeeled sauce after Postharvest Wash Treatments.



1. No wash
2. Water wash
3. 50 ppm chlorine
4. 500 ppm chlorine
5. 10 ppm chlorine dioxide
6. 3 ppm ozone
7. 50 ppm HPAA

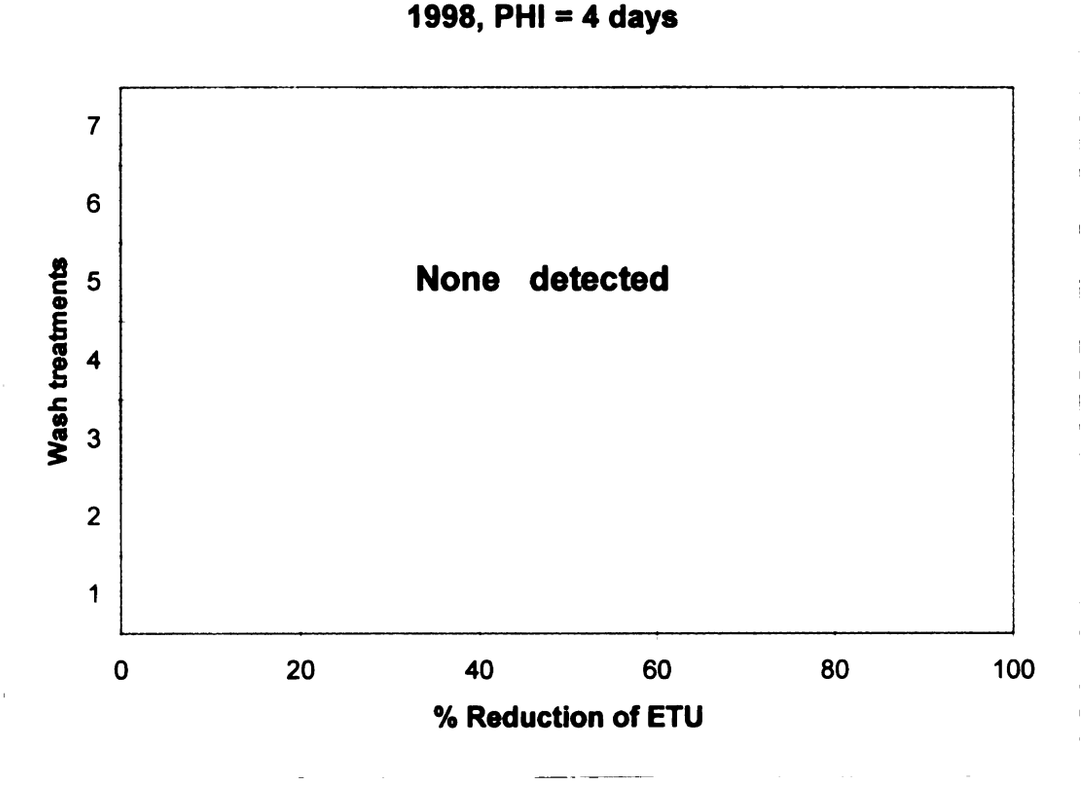


Figure 74. Percent reduction of ETU residues in peeled sauce after Postharvest Wash Treatments.

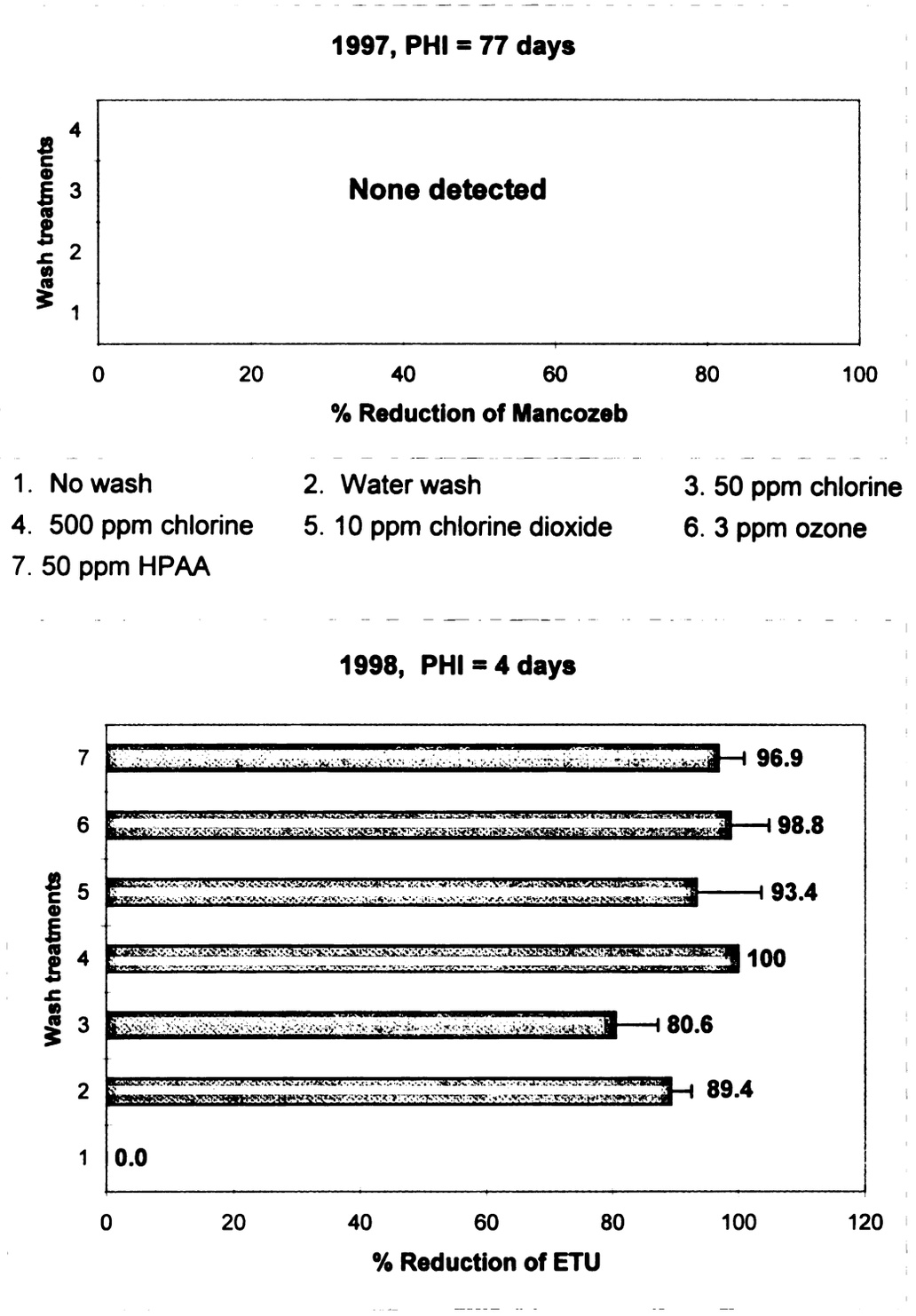
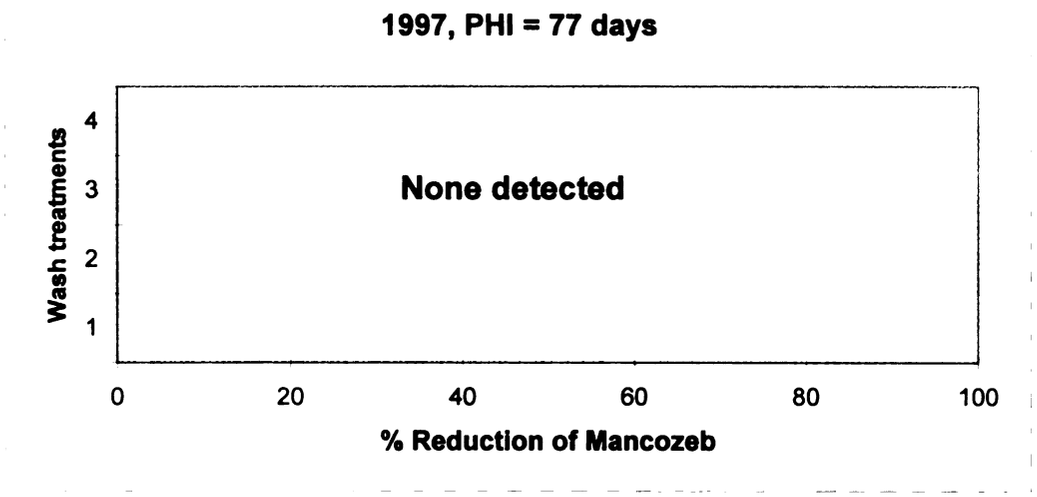


Figure 75. Percent reduction of ETU residues in juice after postharvest wash treatments.



1. No wash
2. Water wash
3. 50 ppm chlorine
4. 500 ppm chlorine
5. 10 ppm chlorine dioxide
6. 3 ppm ozone
7. 50 ppm HPAA

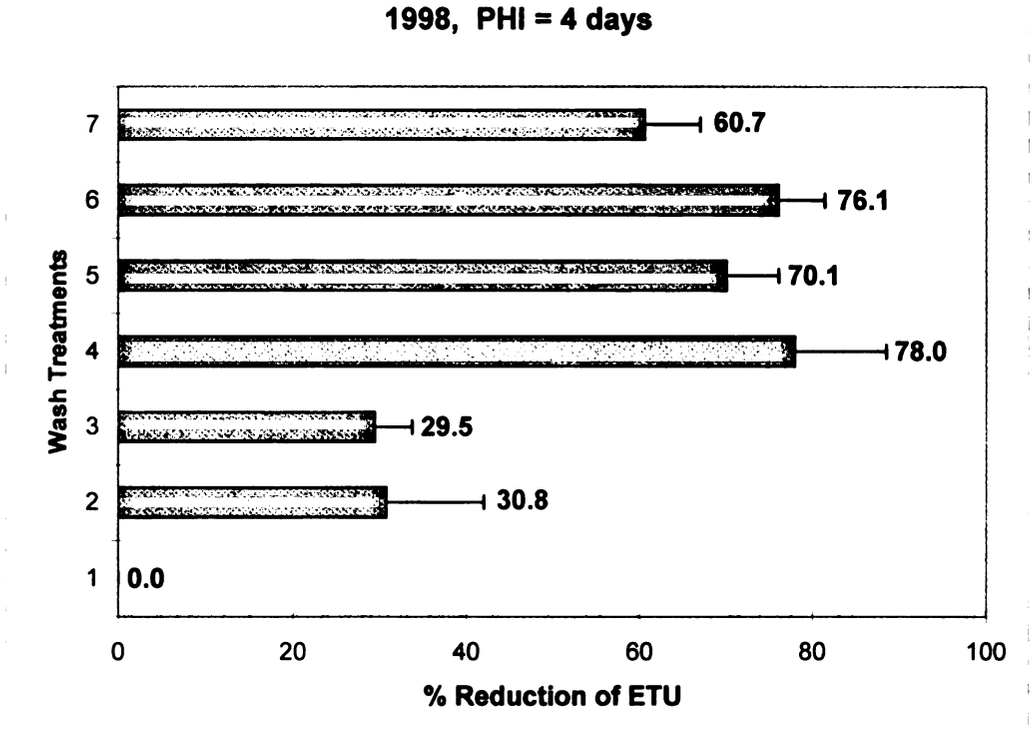


Figure 76. Percent reduction of ETU residues in pomace after postharvest wash treatments.

wash at 50 and 500 ppm removed about 56% and 100% ETU residue, respectively. Apples dipped in chlorine dioxide and HPAA treated water reduced residue levels by about 97%, both. For unpeeled and peeled apple sauce, ETU residue was below the detection limit (Figures 73–74) in 1998 samples. Apple slices and apple juice showed percent reduction of 75–79% and 88–90%, respectively, in water wash only (Figures 72 and 75). In pomace, relatively low levels of percent reduction in ETU residues were detected for all wash-treated samples (Figure 76). This is due to the high concentration of ETU in pomace compared to other products. Even chlorine at 500 ppm and ozone at 3 ppm, gave low percent of reductions with approximately 78 and 76%, respectively.

(III) Comparison of ETU Residue Levels between Products

Reduction of ETU residues by various oxidizing agents showed a pattern similar to mancozeb. High amounts of mancozeb residues resulted in high ETU residues. In unpeeled and peeled apple sauce, no mancozeb was detected. Again, pomace contained high levels of ETU compared to other products for all wash treatments. This indicates that certain processing procedure such as peeling or steaming can play an important role in reducing pesticide residue levels.

SUMMARY & CONCLUSIONS

The objective of the present study was to determine the effects of various wash treatments on the reduction of mancozeb and ETU residues in apples and apple products and determine the effectiveness of different post harvest treatments and processing procedures on the reduction of mancozeb and ETU residues.

Apples sprayed with mancozeb were used to determine the effectiveness of various wash treatments on the removal of the mancozeb and ETU on and in fresh and processed apples. Two–lb apples were used per replication (3 replications per treatment) and placed in a 20 L bucket containing 7 L of water or each oxidizing agent solution. The three treatments in 1997 were (1) No wash, (2) Water wash, (3) Calcium hypochlorite wash @ 50 and 500 ppm and the six treatments in 1998 were (1) No wash, (2) Water wash, (3) Calcium hypochlorite wash @ 50 and 500 ppm, (4) Chlorine dioxide wash @ 10 ppm, (5) Ozone wash @ 3 ppm and (6) Hydrogen peroxyacetic acid wash @ 50 ppm. Mancozeb and ETU residues were analyzed on and in the whole fruit and processed apples using GLC and HPLC.

The amounts of mancozeb residue found on the unwashed whole fruits were below the EPA tolerance level. However mancozeb

detected in pomace was more than EPA tolerance value (3 ppm). Reduction in residual mancozeb was significantly ($p<0.05$) influenced by the effect of various wash treatments as compared to the unwashed samples. There was significantly higher residue in the water washed apples than apples processed with other wash treatments. Chlorine wash at 50 ppm was not especially effective due to its low concentration. Chlorine wash at 500 ppm and ozone at 3 ppm were the most effective treatments for mancozeb and ETU removal in all products. The addition of chlorine, chlorine dioxide, hydrogen peroxyacetic acid, and ozone were shown to be more effective in removing mancozeb residues than a water wash alone. When various wash treatments were combined with processing into apple sauce, mancozeb was reduced by 100% (ie. non-detection levels). Between 48–100% of the mancozeb and 45–100% of the ETU residues were removed after processing. This indicates that certain processing procedure such as peeling or steaming play an important role in reducing pesticide residue levels.

CHAPTER IV. STUDIES ON THE DETERMINATION OF THE DEGRADATION PRODUCTS AND PATHWAYS

INTRODUCTION

Ozone and chlorine dioxide have been widely used for treating drinking water and food processing for many years in many countries. The use of ozone is particularly attractive because it can be applied as a gas or in water, and it dissipates quickly, so that no residue is left on foods (Graham, 1997). Like ozone, chlorine dioxide is a good disinfectant and can kill a large number of microorganisms, including some that are resistant to treatment with chlorine (Richardson *et al.*, 1994). Both these compounds are also being explored for use in reducing pesticide residues on fruits and vegetables and the results have shown them to be effective. However, there is also concern over the presence of chemical by-products that are formed when chlorine, ozone and chlorine dioxide are used for reduction of pesticide residues. Chlorine treatment is known to produce some chemicals that cause cancer in laboratory animals. Use of ozone and chlorine dioxide as alternatives to chlorine for treatment of drinking water and food processing is increasing, mainly because they produce fewer disinfection by-products. Because the alternative disinfectants do not form appreciable levels of these by-products, they are gaining in popularity and use. However, it is unknown whether they produce compounds as harmful or more harmful than those produced by

chlorine. EPA has therefore set out to identify all potentially harmful by–products.

Gas chromatography (GC) is frequently interfaced with mass spectrometry (MS) for confirmation and structural identification of pesticides (Sherma, 1997). Chemical ionization mass spectrometry (CI/MS) is frequently used to generate molecular ions. Electron ionization (EI/MS) is an indispensable tool for determining structures, as it provides the necessary empirical formula information for the molecular ion and fragments. It also helps to limit the number of possible structures for each unknown by–product. GC/IR is useful for determining the functional group (Richardson *et al.*, 1998).

Mass spectrometry is an analytical technique used for the detection of ions and the measurements of their masses, allowing for the identification of the sample. The components become ionized, then travel through a drift region. In the drift region the ions enter a reflectron. By the time the ions reach the detector they are gathered into like–massed groups. The ions hit the detector at the end of their drift. The output of the recording device is a chromatogram. The mass spectrometry process involves three steps: ionization of the sample, mass separation and detection.

A Time–of–Flight Mass Spectrometer (TOFMS) is based on the elapsed time the ion takes from the ion source to the detector. Ions

which have been accelerated to equal energies move with velocities related to their mass–to–charge ratio; these characteristic velocities are used for mass analysis in TOFMS. Ions simultaneously accelerated out of an ion source separate into groups according to their velocities as they travel through an evacuated, field–free tube, as shown in Figure 77. The time elapsed between the extraction of an ion from the source and its detection at the end of the tube is measured and used to calculate mass. In a typical commercial TOFMS instrument, the energy applied for extraction is sufficient to cause ions up to about m/z 1000 to arrive at the detector within 100 μs of the extraction pulse (Yefchak, 1990). The instrument is therefore capable of producing a signal representing 10^4 complete mass spectra each second. This permits analysis of dozens of compounds in 1–3 minutes (Song *et al.*, 1997) due to the extremely rapid spectral acquisition capacity (up to 500 spectra/second) of the mass spectrometer. The use of TOFMS for detection allows compression of chromatography time by permitting significant overlap of eluting compounds without loss of analytical capacity as long as the mass spectra of overlapping compounds differ by a single m/z ratio. In addition, compression of chromatography time results in an increase in sensitivity in that the spectrometer response is concentrated over a shorter time interval than by conventional chromatography. Thus, sampling, chromatographic separation, detection and analysis potentially

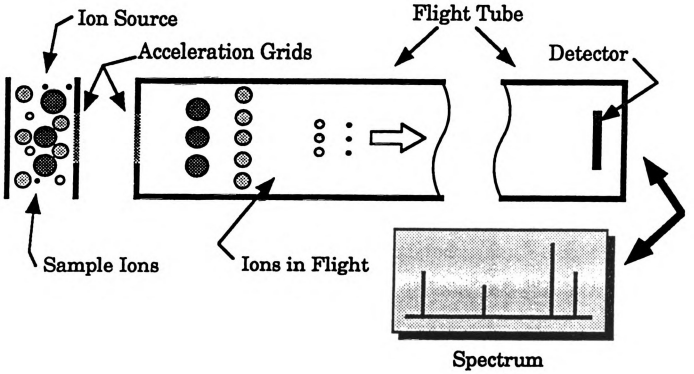


Figure 77. Overall mass analysis process in time-of-flight mass spectrometry.

can be completed in minutes per sample with enhanced sensitivity (Song *et al*, 1998).

Among various oxidizing agents used in Chapters I–III, ozone and chlorine dioxide were selected for this study because they are known to be relatively less toxic and would be good alternatives to chlorine treatment. One objective of this investigation was to determine the by-products of mancozeb and ETU when treated with ozone and chlorine dioxide and elucidate possible degradation pathways of this pesticide. A second objective was to compare our results to previous findings.

MATERIALS AND METHODS

MATERIALS

A. Reagents

(I) Solvents

All organic solvents used for preparation of stock solution, sample extraction and GC/MS were distilled-in-glass grade. Hexane, xylene, chloroform and methylene chloride were obtained from J. T. Baker, Co. (Phillipsburg, NJ).

(II) Standard Chemicals

Mancozeb standard (79.8%) was obtained from Rohm & Hass (Philadelphia, PA). Mancozeb is a complex polymeric, non-crystalline organometallic solid that does not exist in pure form. Standard product material is about 80% pure and contains some stabilizers and formulation materials. Ethylenethiourea (ETU [2–imidazolidinethione], CAS Registry No.96–45–7, 99.0%) and ethyleneurea (EU [2–imidazolidineone], CAS Registry No. 120–93–4, chemical purity 96.0%) standard were obtained from Aldrich Co. (Milwaukee, WI).

B. Glassware

All glassware was thoroughly washed with detergent and warm water, then rinsed with distilled water. The glassware was then rinsed with acetone before being placed in an oven at 400°C overnight before use.

METHODS

A. Ozonation Procedure

A laboratory research ozone generator (Allegheny Teledyne Inc.) was used. Ozone (O_3) was bubbled through a glass sparger (produced bubbles of approximately 10 mm i.d.) into 500 ml of distilled water at ambient temperature and pH under 25 psi at 15 SCFH of oxygen until the desired ozone concentration (3 ppm) was attained. Mancozeb or ETU was spiked to give a final concentration of 100 ppm. After the addition of the mancozeb or ETU, at the desired ozone concentration, the addition of ozone was continued at 25 psi and 15 FCFN of oxygen. A 30 ml sample was transferred at 1, 15, 30 and 60 minute intervals into an Erlenmeyer flask. Two hundred µl of 0.5% 0.1 M sodium thiosulfate solution was immediately added to the samples to quench the reaction.

All ozone concentrations were determined by the method published in Standard Methods for the Examination of Water and Wastewater, 17th Edition (4500–O_3 B Indigo Colorimetric Method, 1987).

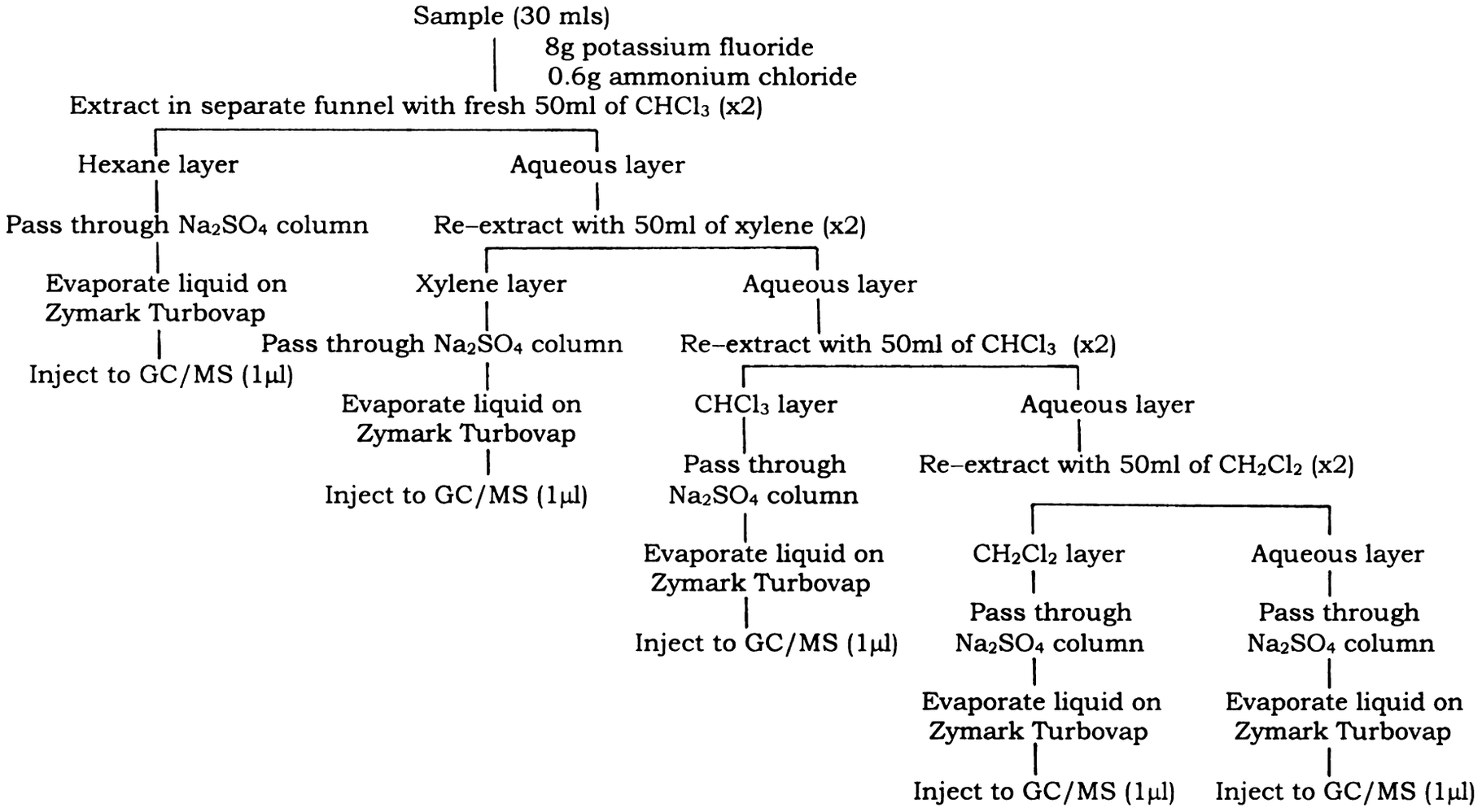
B. Chlorination Procedure

Chlorine dioxide (ClO_2) was generated in the laboratory using the manufacturer's (S.C. Johnson Professional, WI.) instructions as follows. One hundred ml of the stock 2% Oxine FP solution were added to a 200 ml volume French square screw–capped bottle. Twenty five mls of 75% w/w food grade phosphoric acid were added, sealed and allowed to generate chlorine dioxide for 5 minutes with a magnetic stirrer to ensure though mixing. This served as a 100 ppm stock chlorine dioxide solution to achieve the final test concentration. For 20 ppm of chlorine dioxide, added 8 liters of stock solution and made to a total 10 gallon with distilled water. Mancozeb or ETU was spiked to give a final concentration of 100 ppm

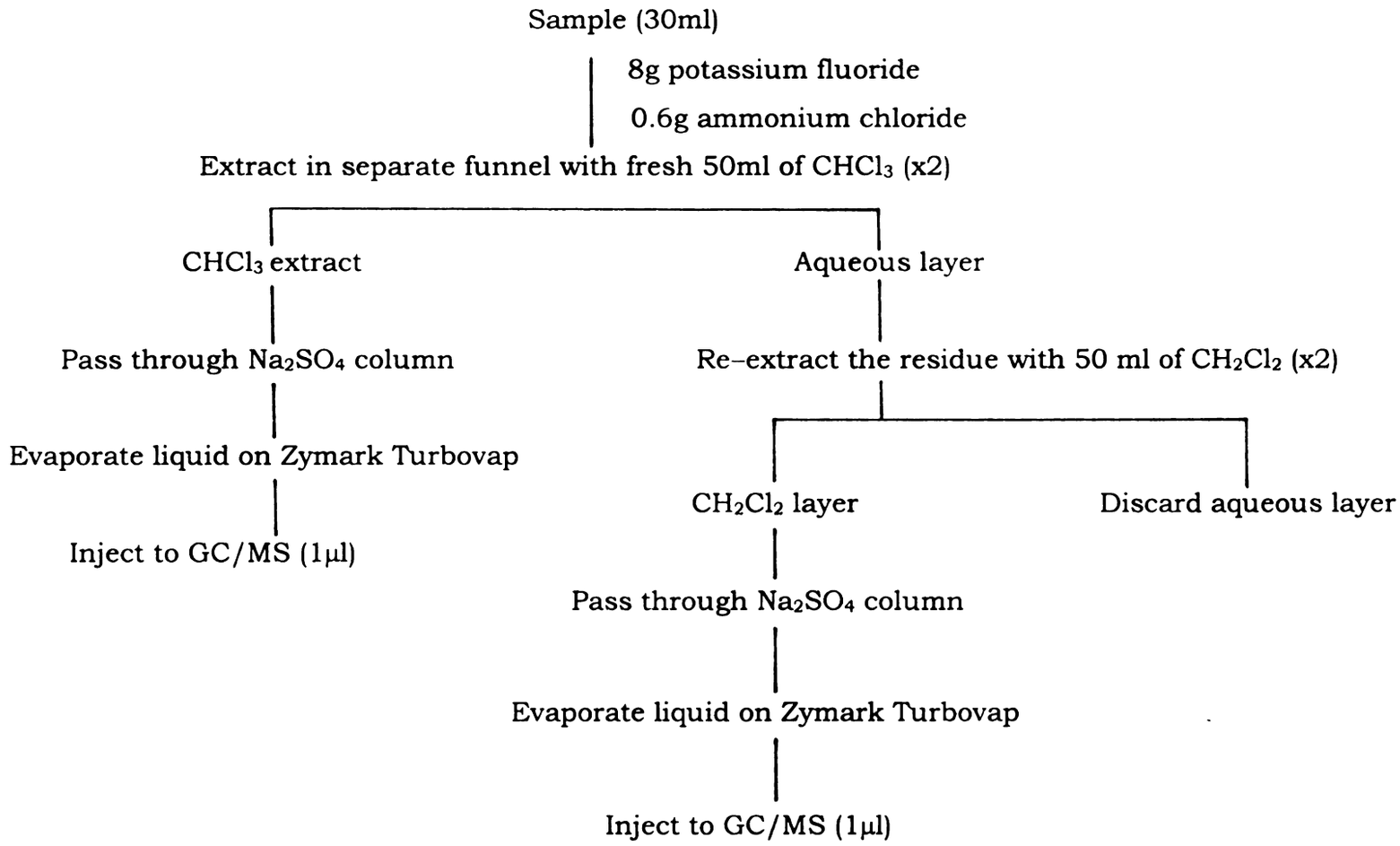
All chlorine dioxide concentrations were determined using the HACH chlorine colorimeter before and after each sampling run. The detailed determination method is given in the methods section of Chapter I.

C. Sample Extraction

Thirty ± 0.1 mls prepared sample were weight in an Erlenmeyer flask, with 8 g of potassium fluoride (KF) and 0.6 g of ammonium chloride (NH_4Cl) and extracted in a 250 ml separatory funnel. In a preliminary study, this mixture was extracted with 5 different solvents according to their polarity. The solvents include hexane (polarity = 7.3), xylene (polarity = 8.8), chloroform (polarity = 9.1), methylene chloride (polarity = 9.6), and water (polarity = 21.0). Scheme 1 shows the diagram of preliminary extraction procedure. From these results, mancozeb and ETU residues were dissolved only in chloroform and methylene chloride layer so these two solvents were used for further extraction procedure. Scheme 2 shows the diagram of revised extraction procedure. The mixture was extracted with 50 ml of chloroform and methylene chloride two times. Then, the solvent layer was passed through a bed of 25 g sodium sulfate (120°C for at least 12 hr) and collected in a Zymark Turbovap tube. The extracted liquid was evaporated to 1 ml at 40°C in a Zymark Turbovap evaporator (Zymark Ind., Hopkin, MA) using nitrogen gas. This reduced extract was determined by GC/MS. By-products of ozone or chlorine dioxide treatment and possible degradation pathways were identified.



Scheme 1. Summary of the extraction method for the analysis of Mancozeb in solution.



Scheme 2. Summary of the extraction method for the analysis of degradation products.

D. GC/MS Analysis

GC/MS analyses was performed on mass spectrometer (LECO Corp., 1997), equipped with a Hewlett Packard Model 6890 gas chromatograph (Hewlett Packard Co., Wilmington, DE) and Pegasus II Version 1.4 computer workstation (LECO Corp., 1997) was used.

Injections of 1 μl of the extract were introduced via a split injector (split ratio=1:10) onto a J & W Scientific hp–5 chromatographic column (30 m, 0.25 mm i.d., 0.25 μm film thickness). Ultrapurified helium (99.999%) was used as carrier gas at a flow rate of 1.5 ml/minute. The GC temperature program consisted of an initial temperature of 40°C, which was held for 1 minute, followed by an increase at a rate of 55°C/minute to 300°C, which was held for 1 minute. Transfer lines were held at 250°C, and the injection port was controlled at 280°C. Sample detection was by Time–of–Flight Mass Spectrometry (TOFMS) with an electron ionization source (FCD–650, LECO Corp, St. Joseph, MI). Mass spectra were collected at a rate of 40/s over the mass range (*m/z*) 33–350. The electron ionization energy was 70 eV. The temperature of the ion source was 200°C.

RESULTS & DISCUSSION

As a first step in this study, five different solvents were selected according to their polarity. These include hexane, xylene, chloroform, methylene chloride and water. In serial extraction, mancozeb and ETU were found in chloroform and methylene chloride layer, so these solvents were selected as a further extraction study. Then ozone and chlorine dioxide treatments of the pesticide were performed and the by-products were identified by GC/MS.

A. By–Products Formed from Hydrolysis

(I) Degradation of Mancozeb

Identification of fragment ions was confirmed by comparison of collected mass spectra with those of authenticated chemical standards and to reference spectra in a mass spectral library (National Institute for Standard Technology, Search Version 1.5, Gaithersburg, MD). A mass spectrum is a graph of ion abundance versus mass to charge ratio. The ions and their abundance serve to establish the molecular weight and structure of the compound being analyzed. Since the ionization process frequently breaks up or fragments the molecule, ions appear in the spectrum at lower m/z values than that which corresponds to the

molecular mass of the molecule. Figure 78 (A) shows a typical spectrum of mancozeb standard at a concentration of 100 ppm, while Figure 78 (B) shows the mass spectrum of the chloroform extract of mancozeb obtained by GC/MS. These spectra corresponded to library search data for mancozeb (Figure 79). In the mass spectrum of chloroform extract, mancozeb has a strong molecular cluster at *m/z* 144, both with and without computer background subtraction (Figure 78(B)). The average retention time of this peak was approximately 181–189 seconds. This corresponded to the ethylene bisdithiocarbamic acid compound minus manganese and zinc ion ($C_4H_4N_2S_2$; 5–Imidazoledithiocarboxylic acid) (Figure 80). Metal ions in mancozeb structure are considered to be very unstable and quickly lost when mancozeb is introduced into high temperature condition. This compound can be present as linear or cyclic form. The major peak with the highest intensity was *m/z* value 72 at 181–181 seconds and several other peaks which include *m/z* 60 and *m/z* 45 appeared. The ion at *m/z* 85 carried a smaller portion of the total ion current. The fragment ions were used to determine molecular structure. The proposed structures of the fragment ions are illustrated in Figure 81.

(II) Degradation of ETU

Figure 82 (A) shows a typical spectrum of ETU standard at a concentration of 100 ppm, while Figure 82 (B) shows the mass spectrum

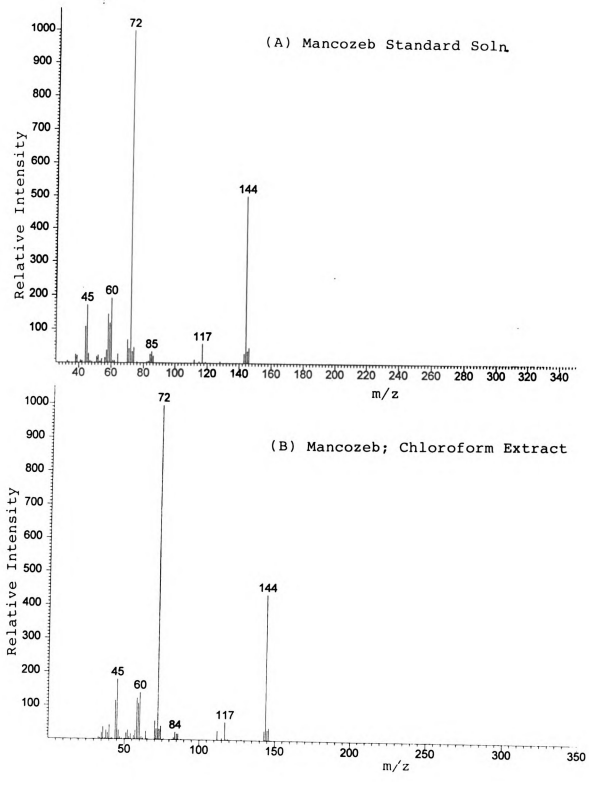


Figure 78. A typical spectrum of mancozeb from (A) standard solution at 100 ppm in distilled water and (B) chloroform extract.

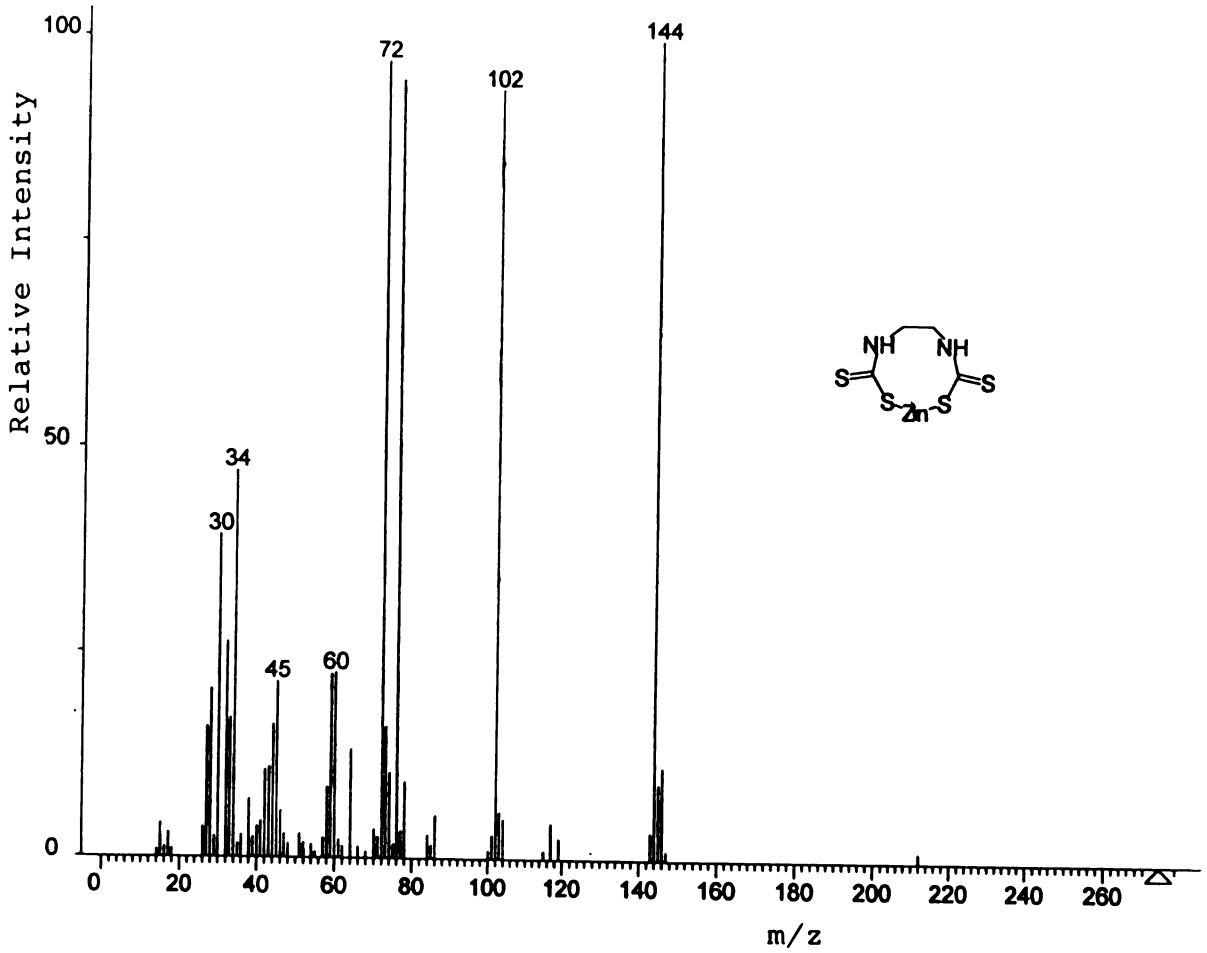


Figure 79. The mass spectrum of Mancozeb obtained from library search.

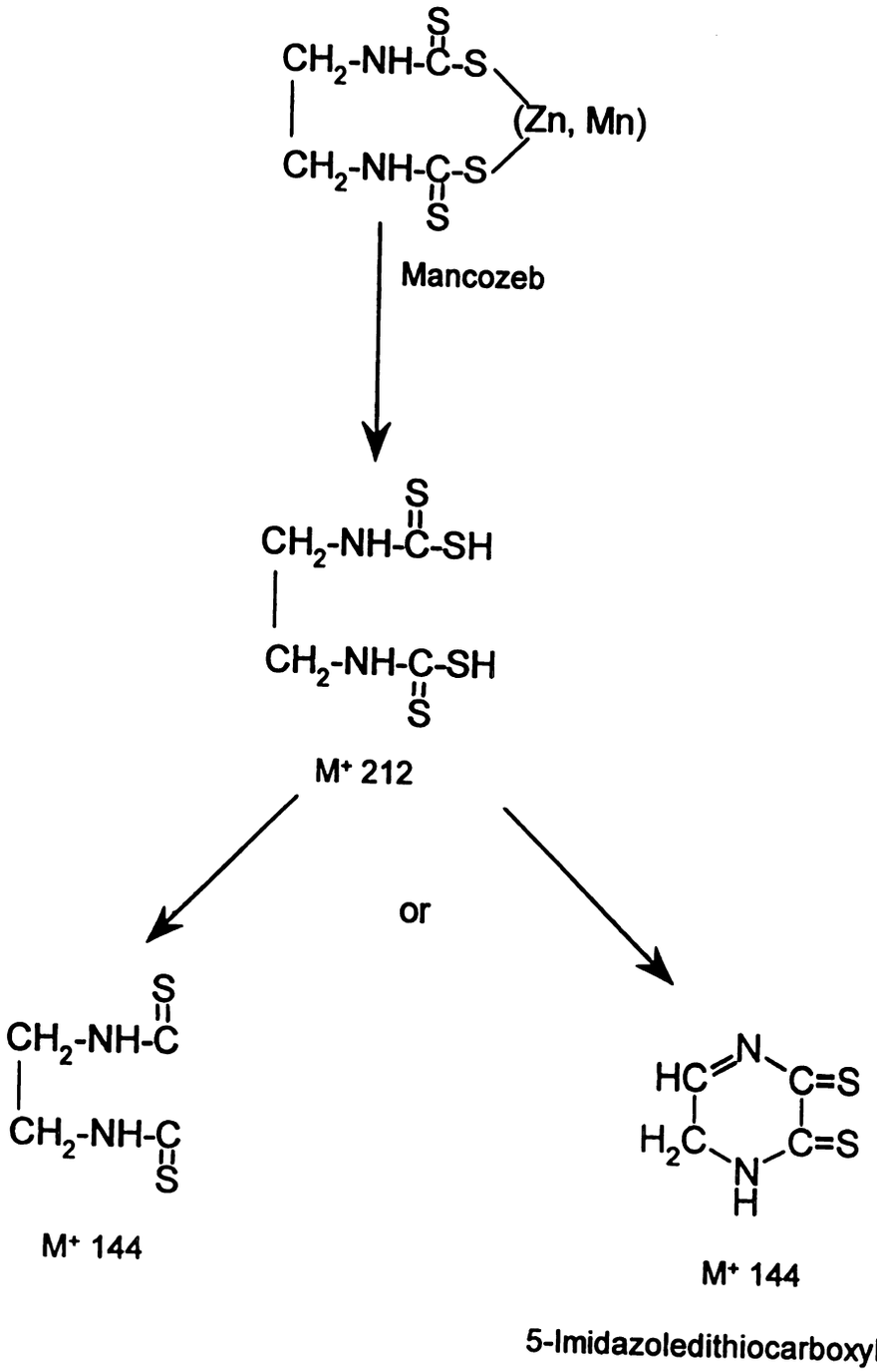


Figure 80. Possible fragmentation of Mancozeb by hydrolysis.

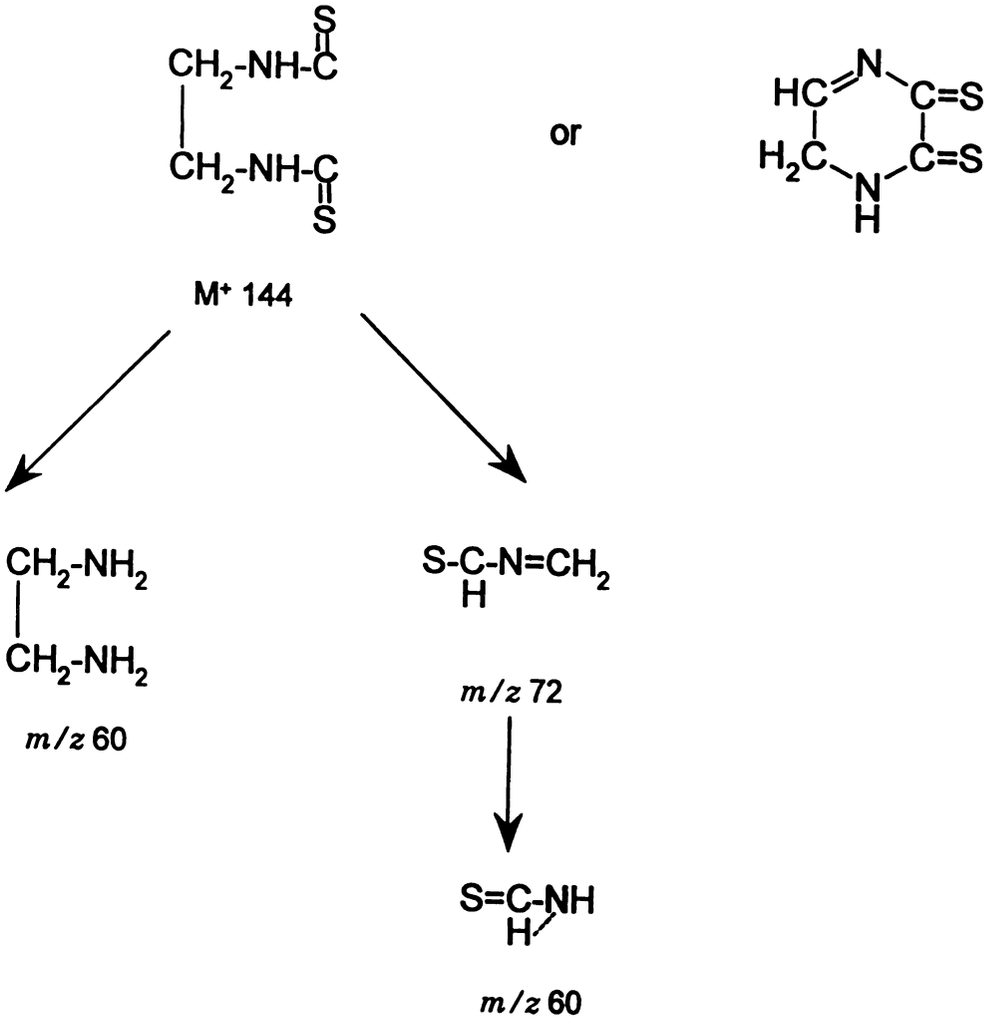


Figure 81. Proposed degradation pathway of Mancozeb in aqueous solution by hydrolysis.

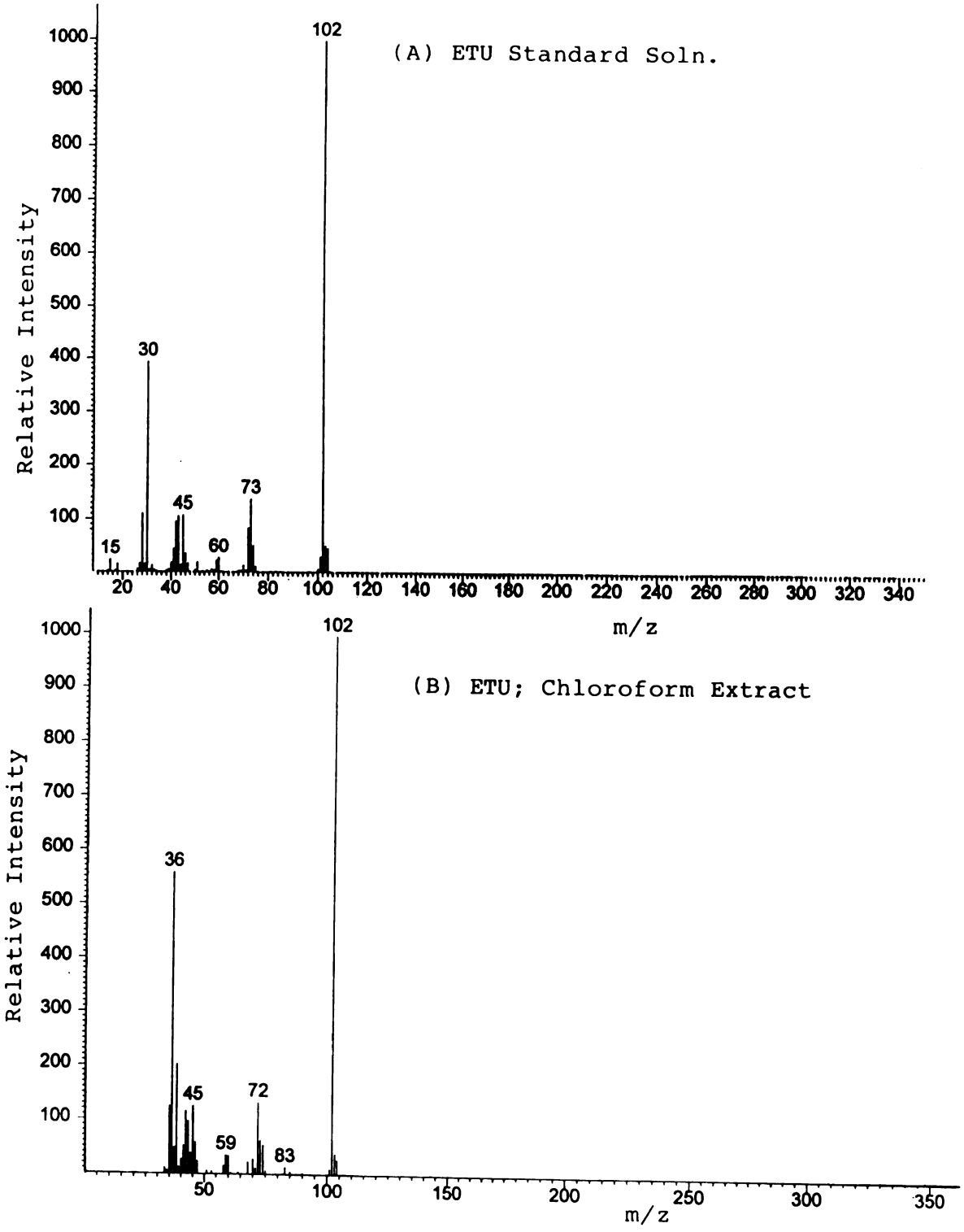


Figure 82. A typical spectrum of ETU from (A) standard solution at 100 ppm in distilled water and (B) chloroform extract.

of chloroform extract of ETU obtained by GC/MS. These spectra corresponded to library search data for ETU (Figure 83). After 60 minutes reaction in distilled water, the spectrum showed similar patterns to that of 0 minutes and still had a strong molecular cluster at *m/z* 102 (Figure 84). The M^+ (102) corresponded to molecular weight of ETU. This indicates that ETU was stable in distilled water and did not undergo hydrolysis during 60 minutes. The average retention time of ETU was approximately 210–230 seconds.

(III) Effect of pH on the Formation of Mancozeb Degradation Product

The mass spectra of mancozeb in each pH solution were collected and monitored for a period of sixty minutes at both chloroform and methylene chloride layers. Chloroform layer showed more intensive GC/MS response to the mancozeb degradation products than methylene chloride layer. This was due to the effect of serial extraction. Most mancozeb residues were extracted by chloroform and only small amounts of mancozeb residues remained on the methylene chloride layer. In pure mancozeb standard solution, the most abundant ion was *m/z* value 72. In Figure 85–86, the time dependence of the GC/MS response as the peak area of the molecular ion (M^+ 72) is shown. As time elapsed the relative response of the ion currents at *m/z* values 72 increased in

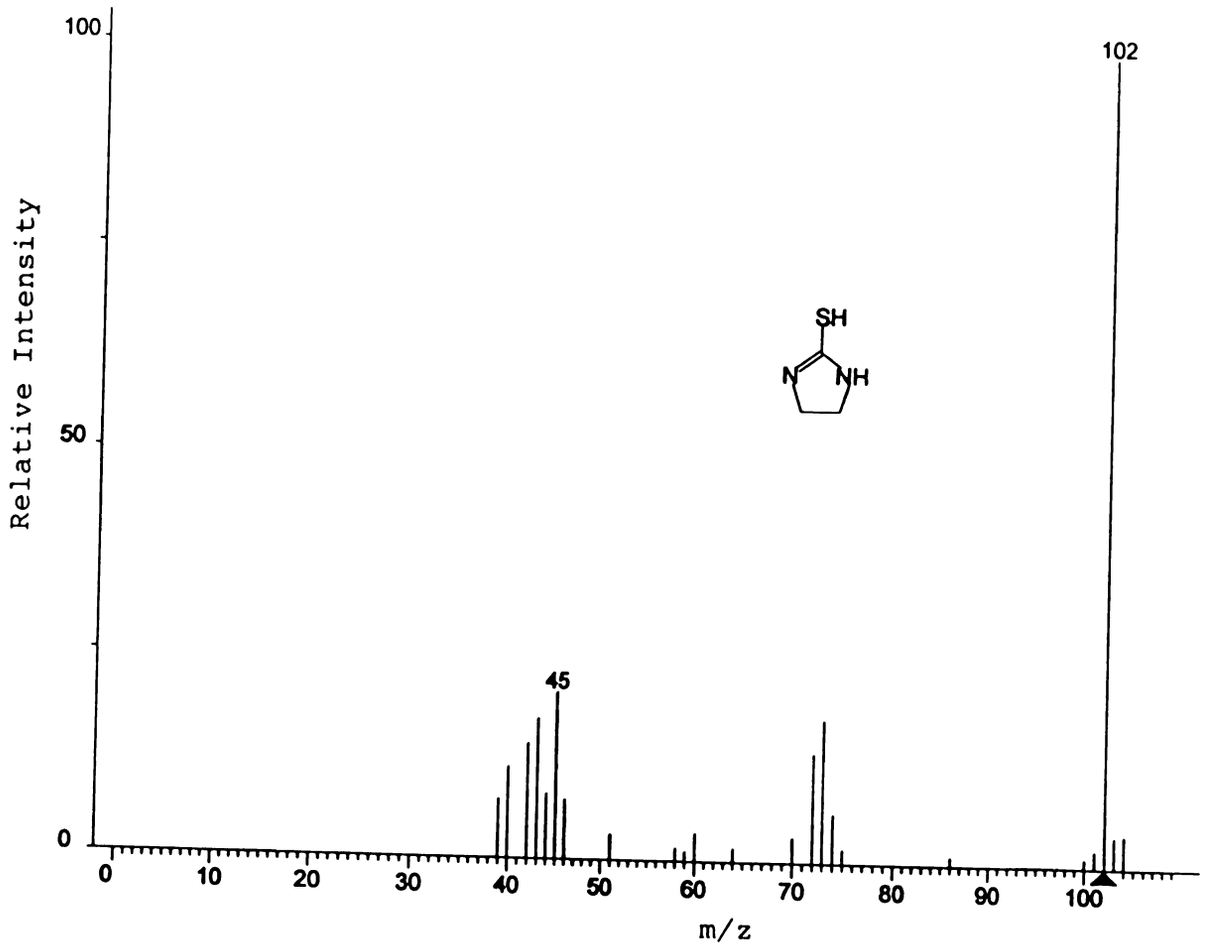


Figure 83. The mass spectrum of ETU obtained from library search.

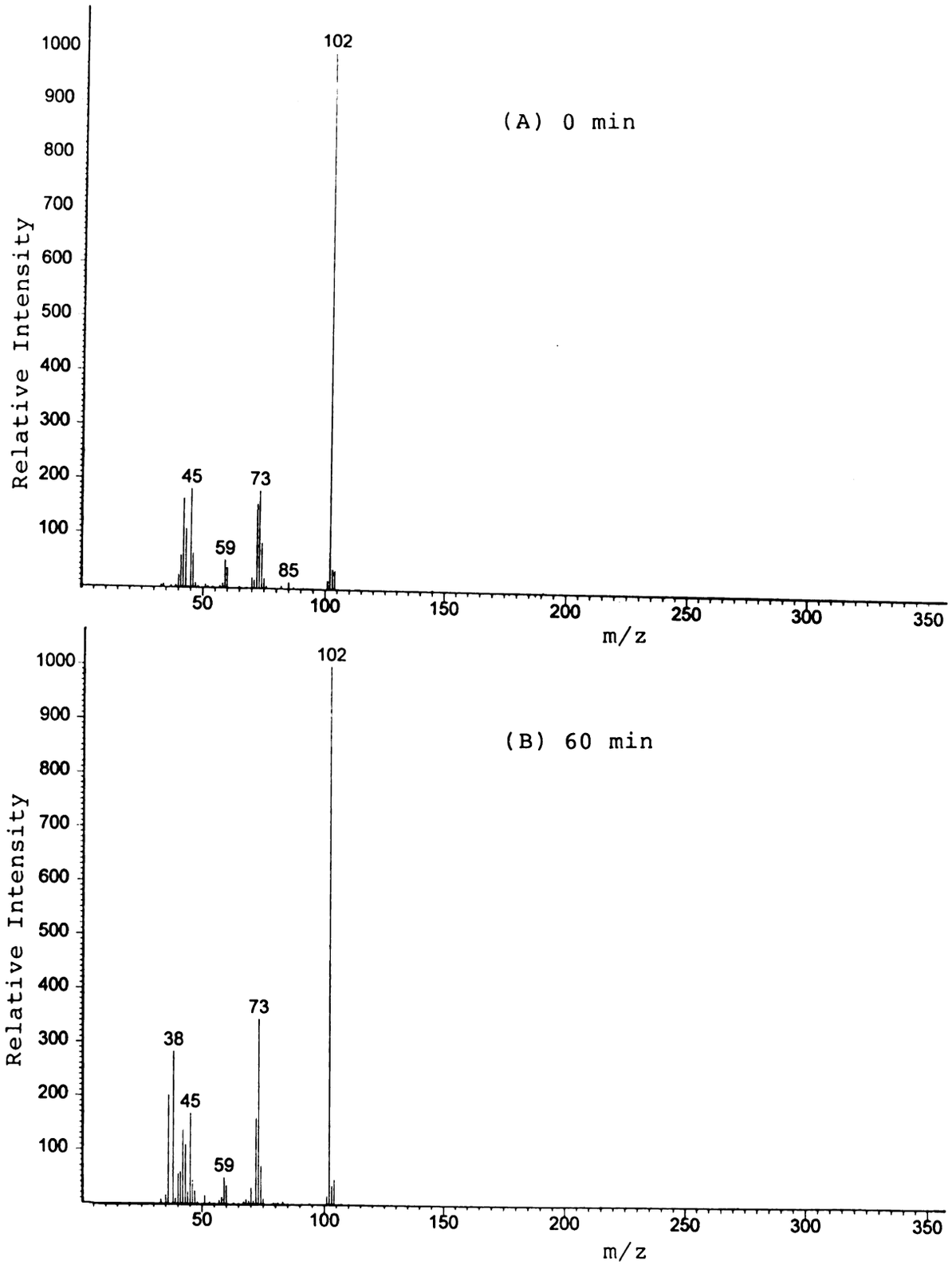


Figure 84. The mass spectrum of ETU obtained from chloroform extract at (A) 0 minute and (B) 60 minute reaction time in distilled water.

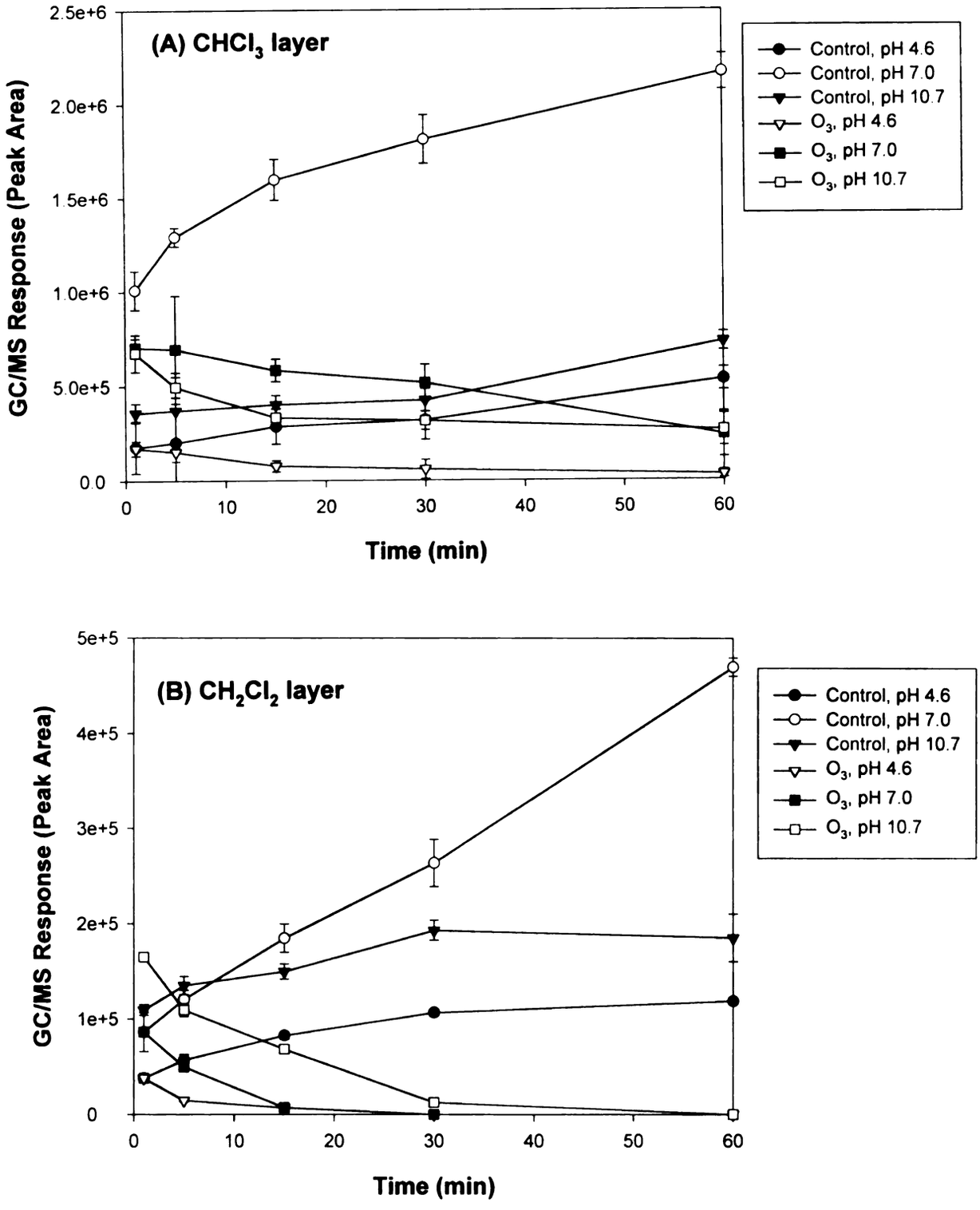


Figure 85. Effect of ozone on time dependence of the GC/MS response on the formation of molecular ion (M^+ 72) from (A) $CHCl_3$ layer and (B) CH_2Cl_2 layer.

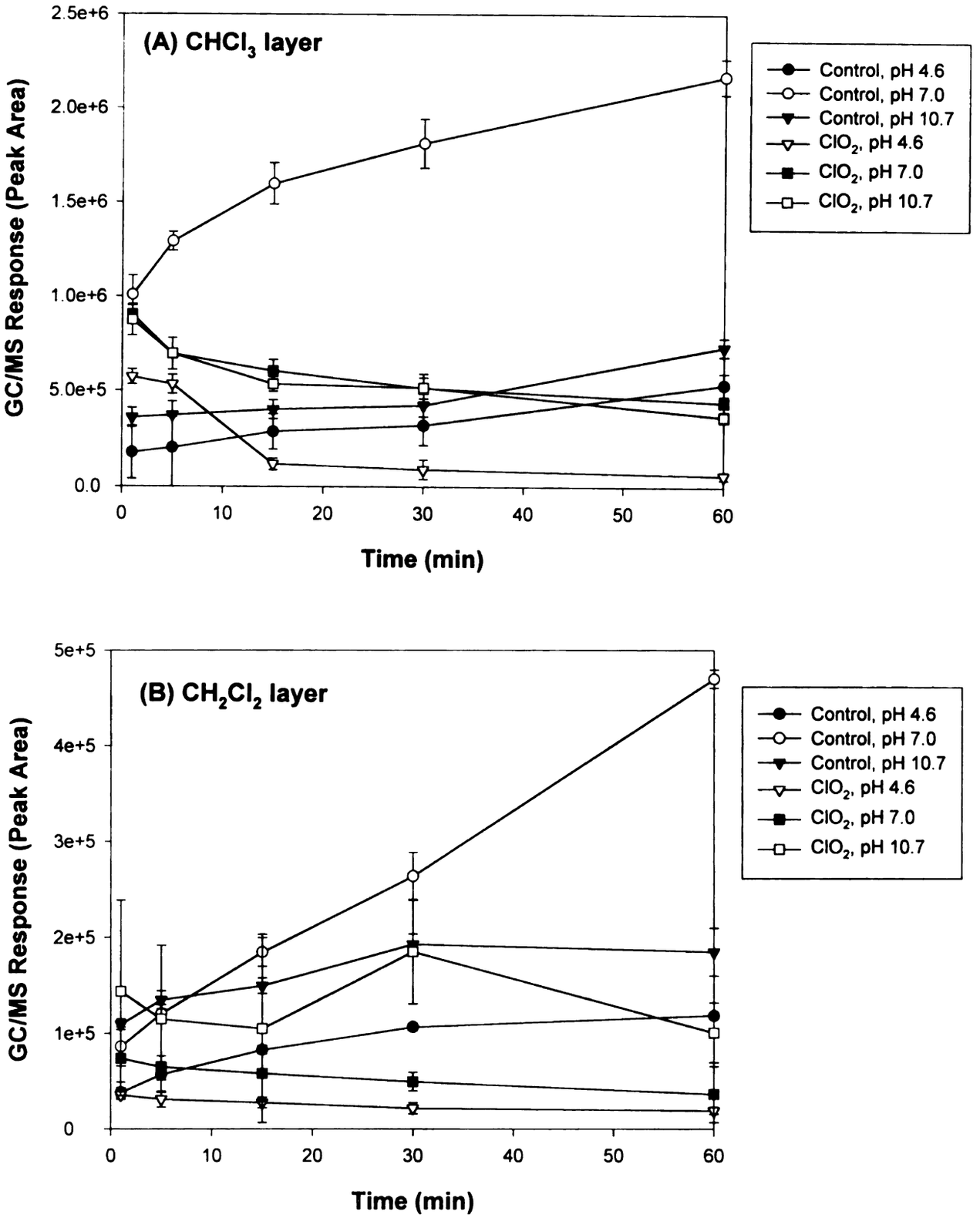


Figure 86. Effect of chlorine dioxide on time dependence of the GC/MS response on the formation of molecular ion (M^+ 72) from (A) $CHCl_3$ layer and (B) CH_2Cl_2 layer.

control treatment at three pH ranges. The formation of *m/z* 72 was the greatest at pH 7.0 and decreased in pH 4.7 and pH 10.7. This result suggest that the *m/z* 72 ion was stable at neutral pH and the formation of this ion increased as time elapsed.

Ozone treatment at pH 4.6 showed preventive effect on the formation of *m/z* 72 ion (Figure 85). The ozone treatment at pH 10.7 was the least effective. No *m/z* 72 ion was detected at pH 4.6 or pH 7.0 after 60 minutes reaction time. This was due to the instability of ozone at alkaline condition. These results corresponded to the model system study. Chlorine dioxide also showed preventative effect on the formation of *m/z* ion (Figure 86). pH 4.6 showed the most effectiveness and pH 10.7 was the least effective in both chloroform and methylene chloride layer. However, the effect was lower than ozone treatment. *m/z* 72 ion still remained at 20 ppm chlorine dioxide treatment after 60 minutes.

B. By-Products Formed from Ozonation

(I) Degradation of Mancozeb

Ozonation of mancozeb produced ETU, with a retention time of 206 seconds. When the reaction between mancozeb and ozone continued, degradation of mancozeb occurred. At 30 minutes reaction time, the total amount of *m/z* 144 ion decreased compared to 1 minute. After 60 minutes ozone treatment, no *m/z* 144 was detected at 206

seconds (Figure 87). Oxidation due to ozonation or hydrolysis changes the by–products into high polarity hydrophilic compounds, such as ETU and others. Analysis of the aqueous ozonation of mancozeb and its degradation products demonstrated that metal groups, such as manganese and zinc, are the first site of attack and the CS_2 or CS group was removed.

Usually, reference standards are pure compounds, however the sample extracts are not, so they can introduce interfering ions into the mass spectrum, complicating the confirmation process. Mancozeb is a complex polymeric, non-crystalline organometallic solid that does not exist in pure form. Standard mancozeb is about 80% pure and contains some stabilizers and formulation materials. So, determination of some oxidation products was not possible because of matrix interference.

(II) Degradation of ETU

Treatment of ETU with ozone yielded several degradation compounds. Figure 88 presents the total ion current (TIC) of ETU obtained from chloroform layer with 3 ppm ozone treatment after 60 minutes. Prolonged ozonation (60 minutes) of ETU eventually gave rise to EDA (ethylenediamine) and several degradation products but no ethyleneurea (EU) was detected in this study. The mass spectrum of each molecular ion (M^+) used to determine the possible degradation products

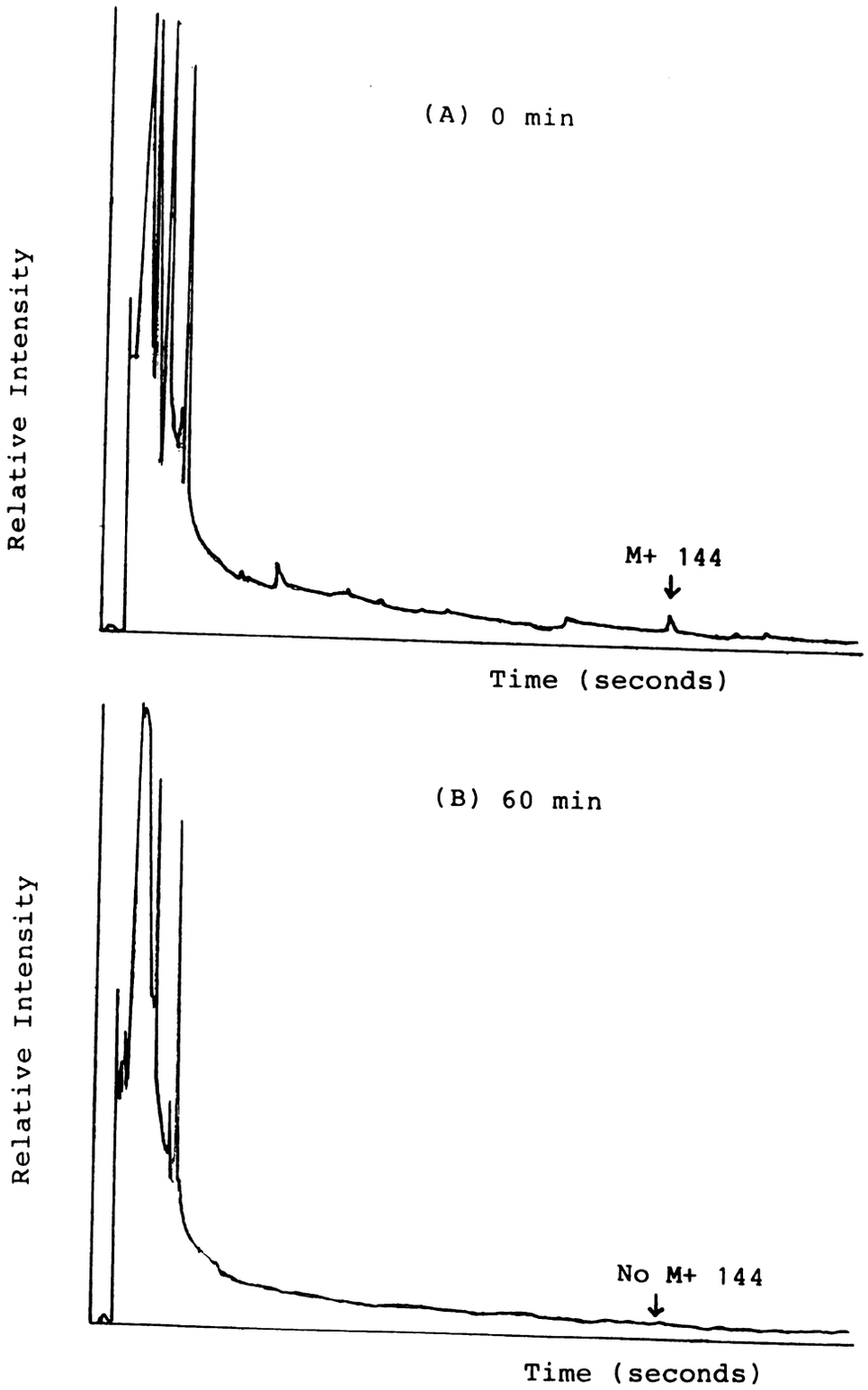


Figure 87. Comparison of chromatogram of Mancozeb at (A) 0 minutes and (B) 60 minutes in ozone treatment.

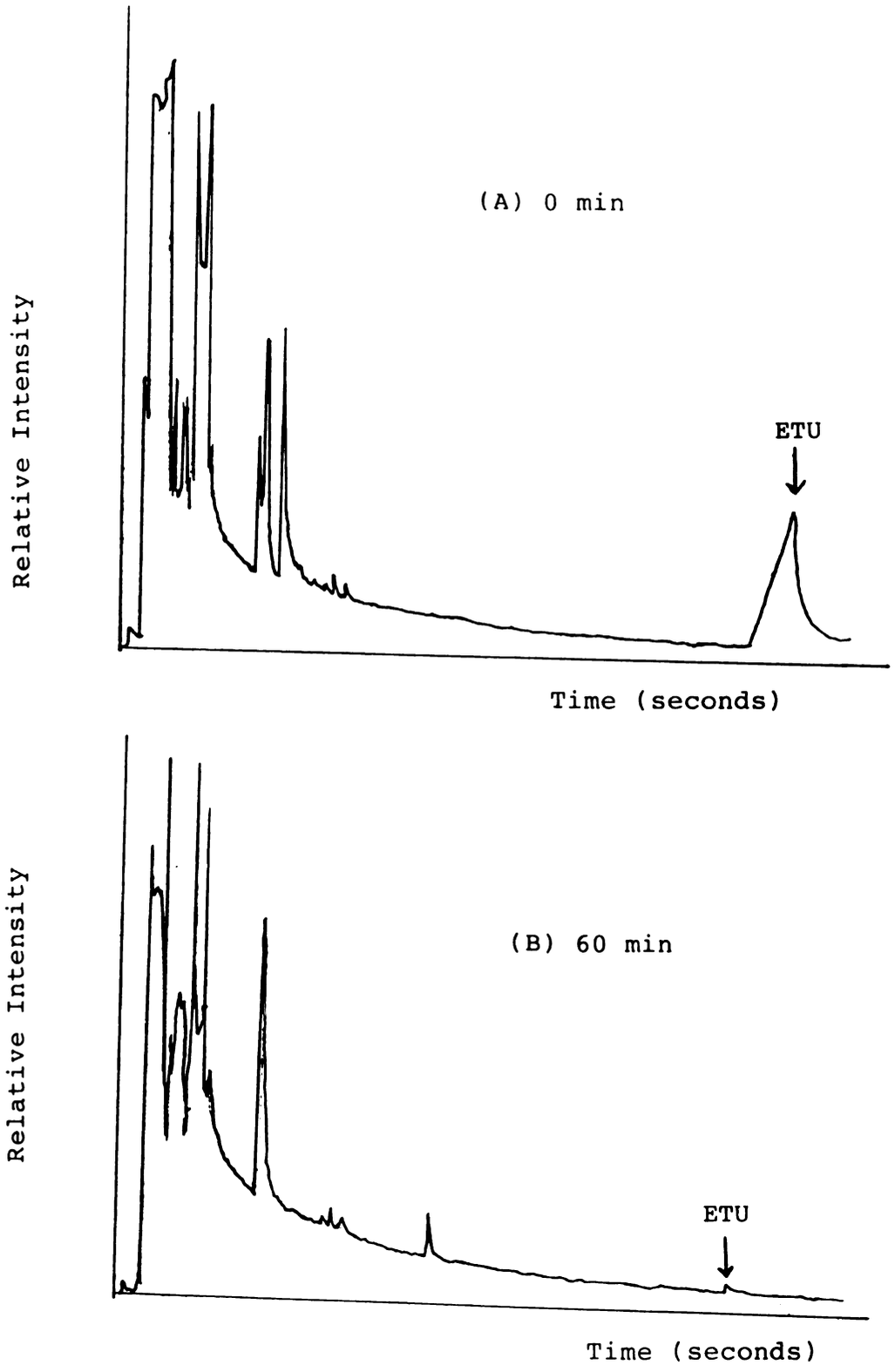


Figure 88. Total ion current (TIC) of ETU in ozone treatment at (A) 0 minutes and (B) 60 minutes in chloroform extract.

are shown in Figure 89. The molecular ions found as ETU degradation products by ozone treatment were M^+ 60 at 42.77 seconds, M^+ 84 at 47.87 seconds, M^+ 163 at 61.37 seconds, M^+ 117 at 62.47 seconds and M^+ 267 at 131.57 seconds. The proposed structures of the degradation products are illustrated in Figure 90. The degradation by-products were confirmed with previous findings (Aizawa, 1991). The results suggest that ozonation increases the removal of ETU and produce several degradation products. These results, however, do not reveal the underlying mechanism(s) or toxicity. Hence, more detailed studies are required in order to identify these mechanisms and subsequently, optimize the combined treatment process. Toxicity tests are also required.

C. By–Products Formed from Chlorine Dioxide

(I) Degradation of Mancozeb

Mancozeb with chlorine dioxide treatment produced ETU, with a retention time of 206–218 seconds. When the reaction between mancozeb and chlorine dioxide continued, the degradation of mancozeb occurred. At 30 minutes reaction time, the total amount of *m/z* 144 ion decreased as compared to 0 minutes. After 60 minutes chlorine dioxide treatment, small peak of *m/z* 144 was still detected. This indicates that mancozeb residue did not completely degrade into other by products but still remained. This was probably due to the high concentration of

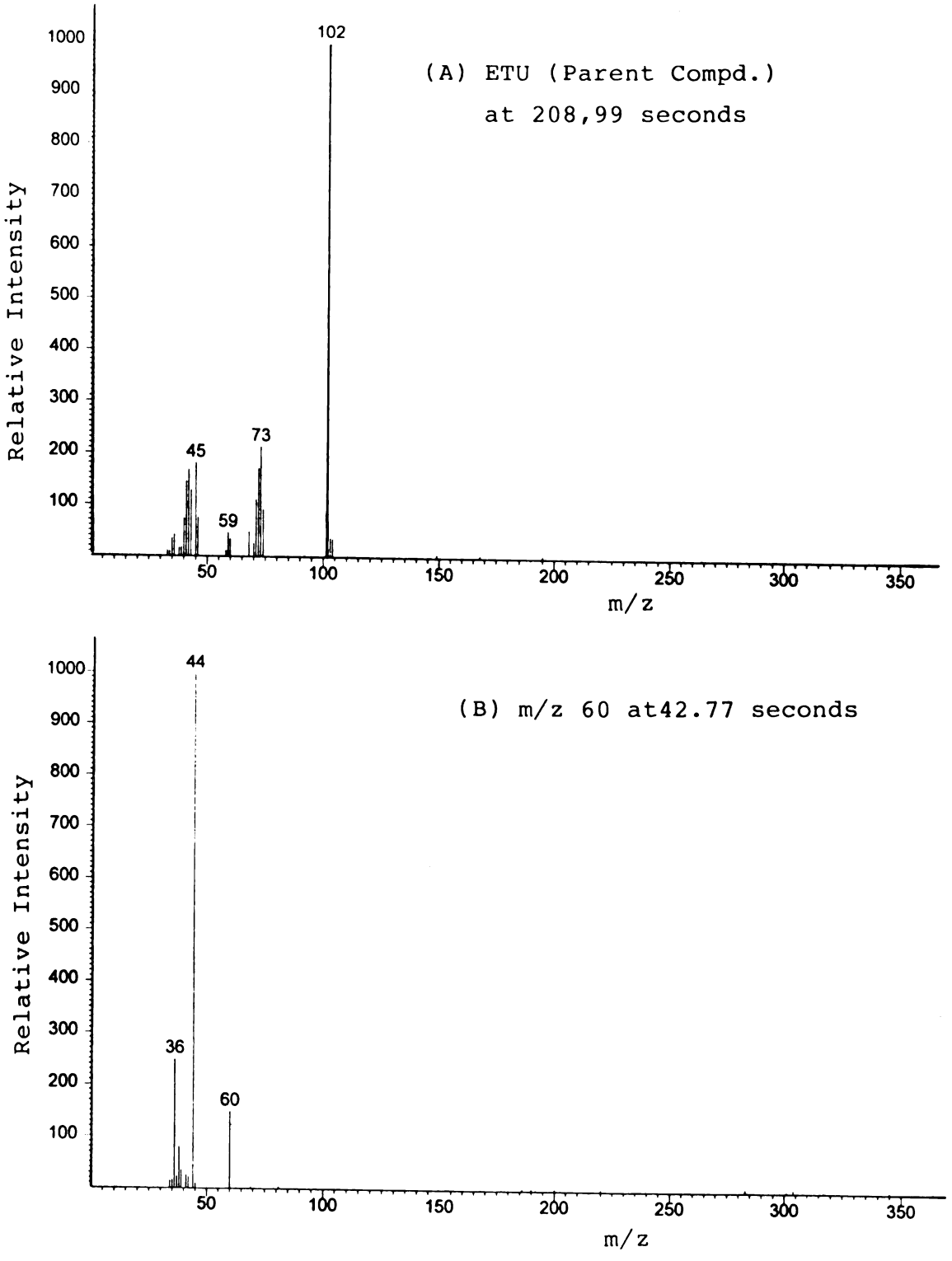


Figure 89. The molecular ions found as ETU degradation products by Ozone (Cont'd).

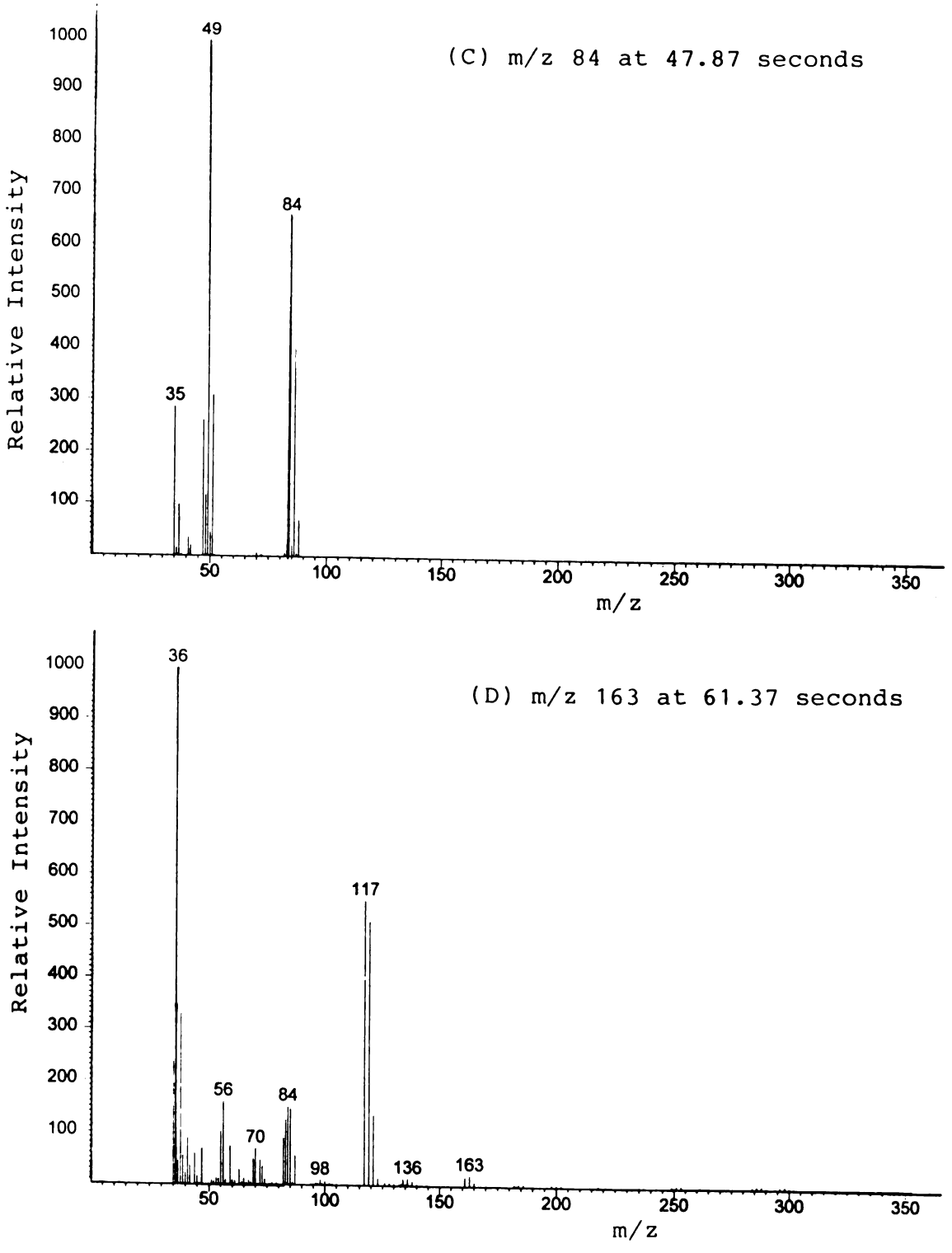


Figure 89. The molecular ions found as ETU degradation products by Ozone.

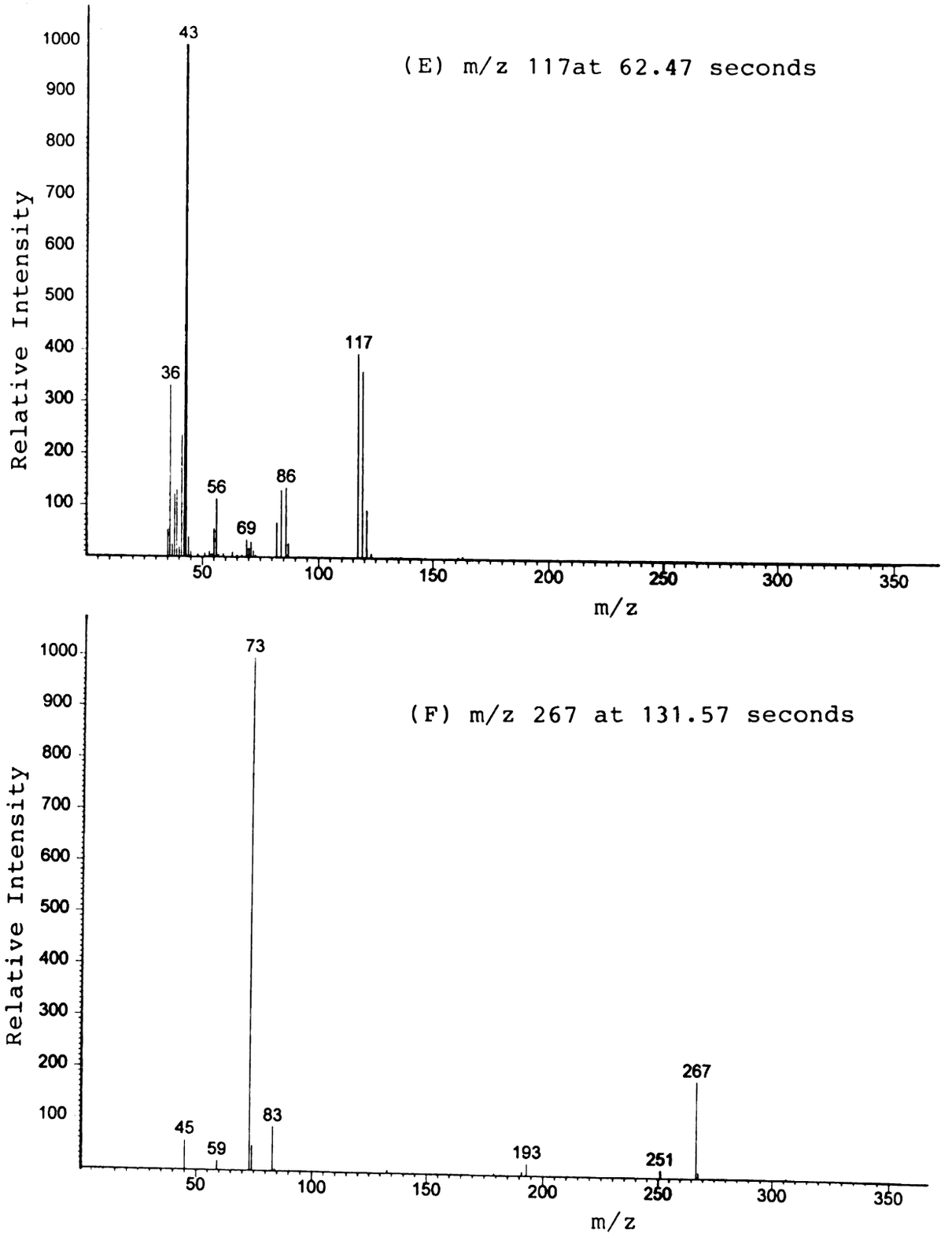


Figure 89. The molecular ions found as ETU degradation products by Ozone.

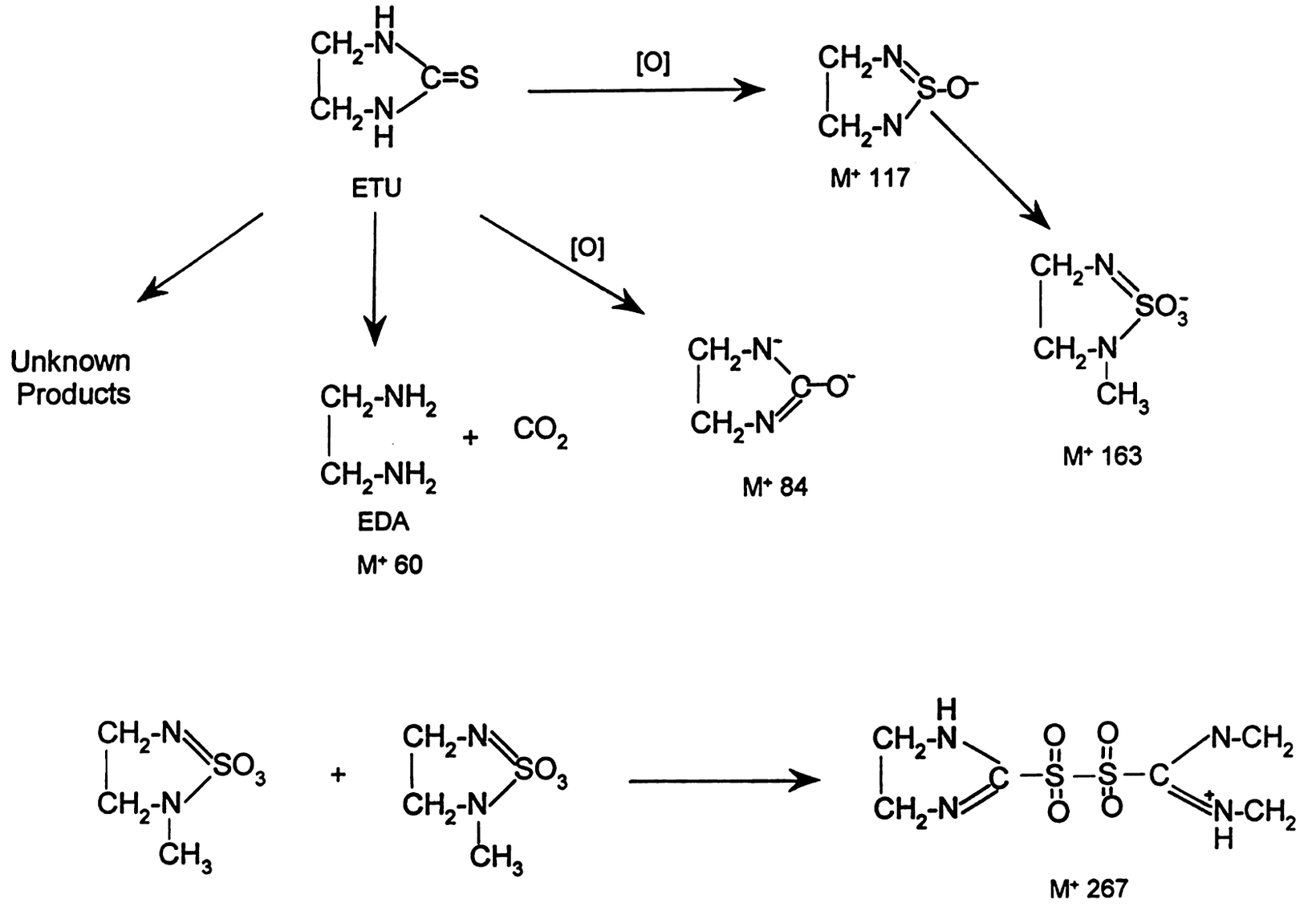


Figure 90. Proposed degradation pathway of ETU in ozonation.

mancozeb (100 ppm) compared to low chlorine dioxide concentration. It is anticipated that *m/z* 144 peak would completely disappear with chlorine dioxide treatment if the concentration of chlorine dioxide is increased above the 20 ppm that was used in this study.

(II) Degradation of ETU

Treatment of ETU with chlorine dioxide yielded several degradation compounds. Figure 91 presents the total ion current (TIC) of ETU obtained from chloroform layer with 20 ppm chlorine treatment after 60 minutes. At prolonged ozonation (60 minutes), ETU was oxidized to ethyleneurea (EU) at a retention time of 162–180 seconds. However, ETU was still detected at 209–221 seconds in the spectra. This mean that ETU did not completely degrade into other by products but still remained in the reaction mixture. This was probably due to the high concentration of ETU (100 ppm) compared to low chlorine dioxide concentration. The mass spectrum of each molecular ion (M^+) used to determine the possible degradation products of chlorine dioxide treatment are shown in Figure 92. The molecular ions found as ETU degradation products were M^+ 117 at 62.72 seconds, M^+ 86 at 160.12 seconds and M^+ 163 at 61.37 seconds. Several unknown products are also present. The proposed structures of the degradation products are illustrated in Figure 93. Chlorine dioxide showed less effectiveness in

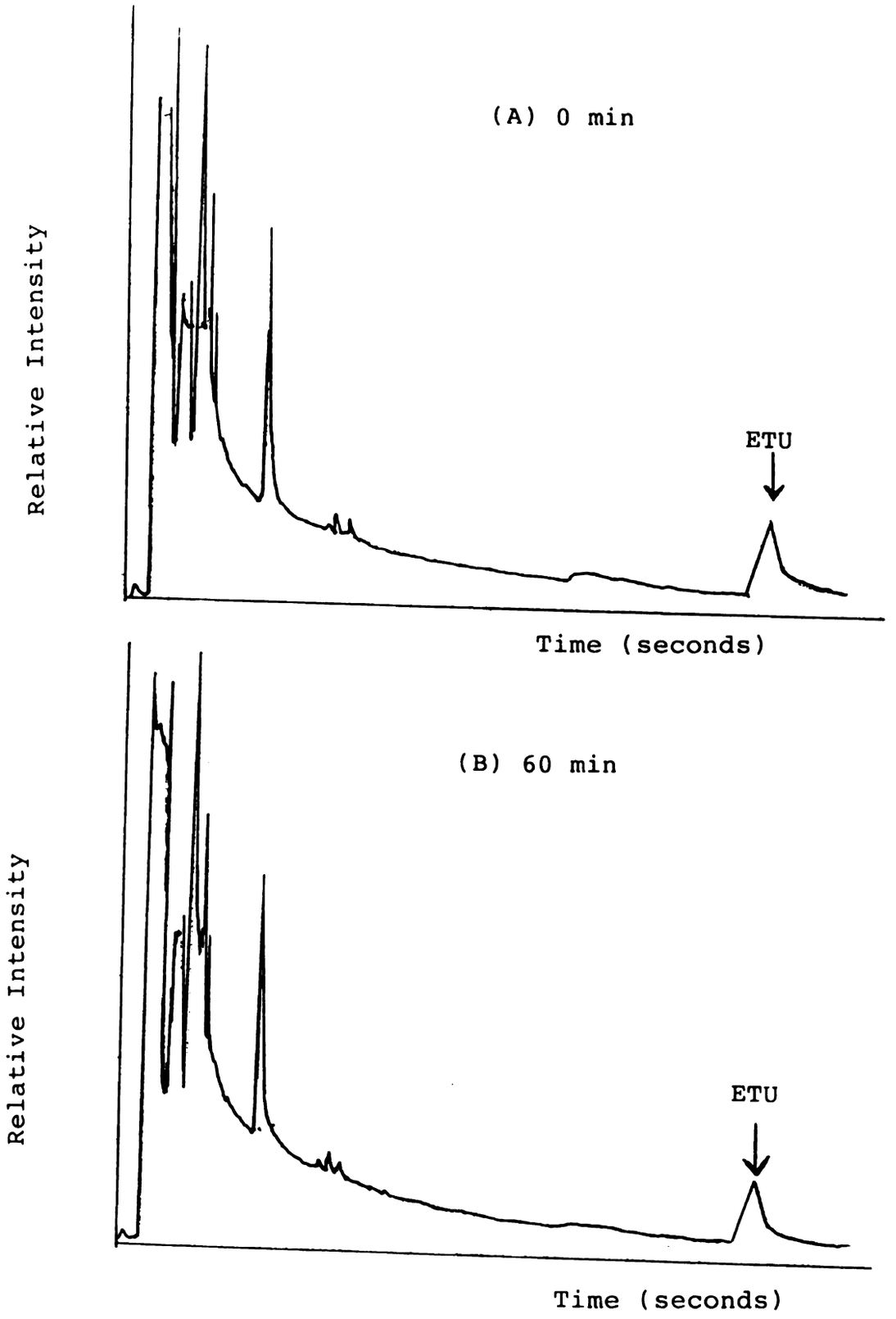


Figure 91. Total ion current (TIC) of ETU in chlorine dioxide treatment at (A) 0 minutes and (B) 60 minutes in chloroform extract.

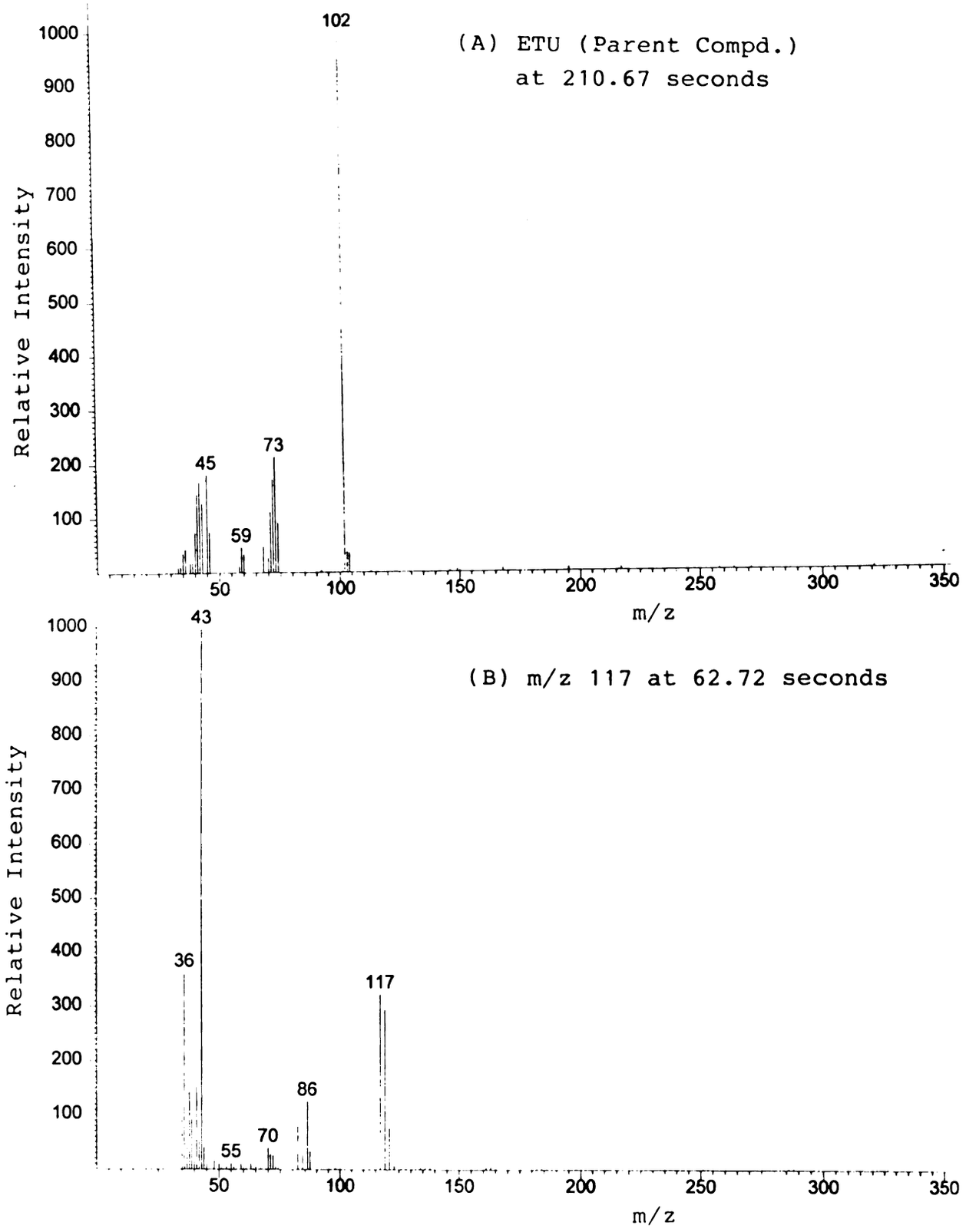


Figure 92. The molecular ions found as ETU degradation products by Chlorine Dioxide (Cont'd).

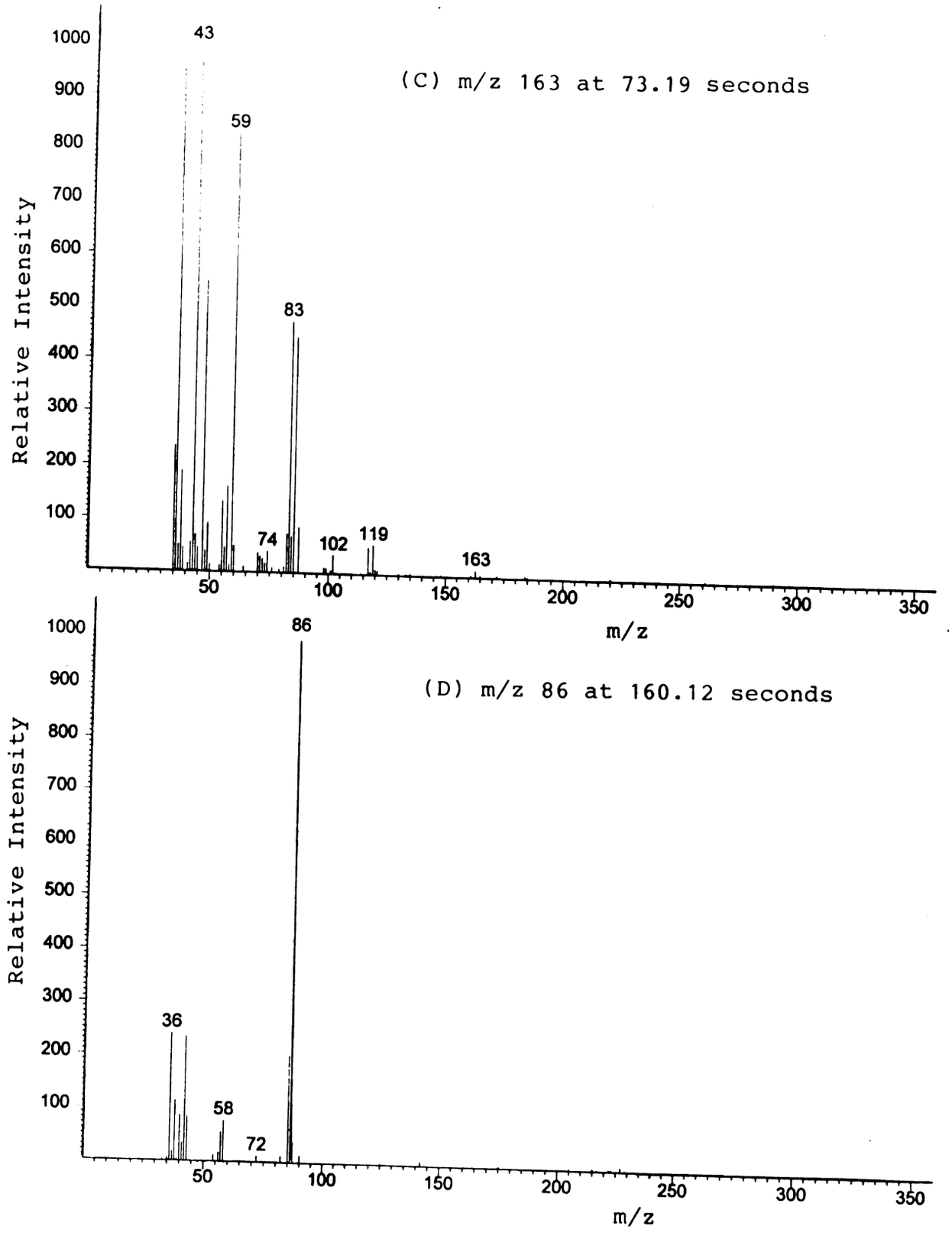


Figure 92. The molecular ions found as ETU degradation products by chlorine dioxide.

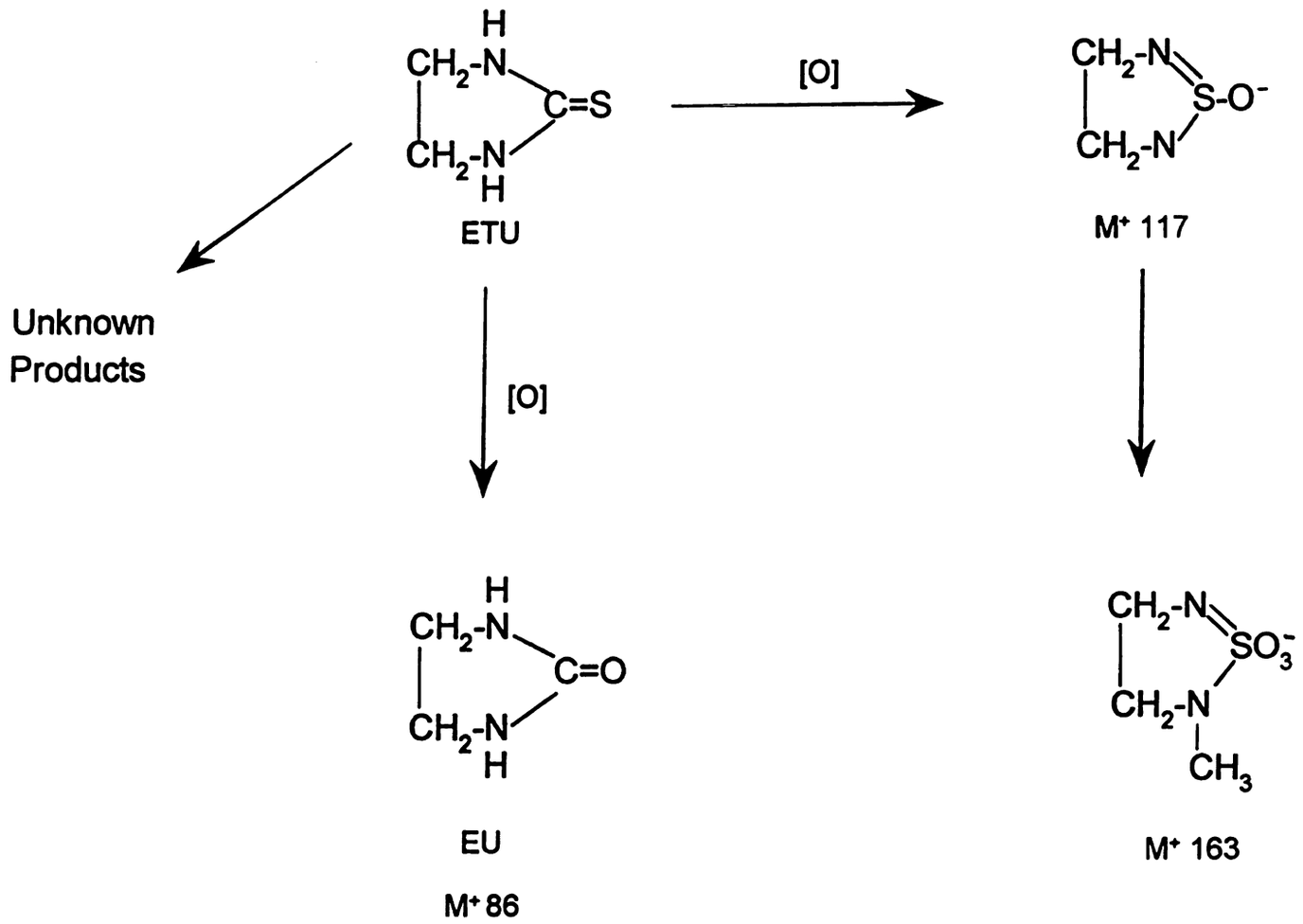


Figure 93. Proposed degradation pathway of ETU by chlorine dioxide.

degradation of ETU compared to ozone treatment. ETU was produced less degradation products compared to ozonation. This is probably due to the fact that ETU was not completely degraded by chlorine dioxide.

The results suggest that low dose chlorine dioxide treatment does not significantly remove mancozeb and ETU. However, the effect of chlorine treatment may be expected to depend on the applied chlorine dioxide dosage, contact time, as well as the concentration of mancozeb present in solution. Consequently, further studies are required in order to assess these effects.

Overall, many by–products were identified, several of which have never been reported previously. Many of the compounds were not present in any spectral library (NIST or Wiley), and many of the ones that were in the libraries did not give conclusive library matches (Richardson *et al.*, 1998). For many of the compounds, little information was provided by the mass spectra, because of the absence of molecular ions, which provide molecular weight information.

SUMMARY & CONCLUSIONS

The objective of the present study was to determine the degradation products of mancozeb and ETU and elucidate the possible degradation pathways in solutions as a result of chemical oxidation using ozone and chlorine dioxide. This study was developed in a solution at 100 ppm mancozeb and ETU concentration during 60 minutes. Two different oxidizing agents used in this study were (1) Ozone @ 3 ppm and (2) Chlorine dioxide @ 20 ppm. Ozone was continuously provided throughout the course of the reaction. Degradation products were detected with high resolution GC/MS. The total analysis time was 4 minutes per sample combined with rapid gas chromatographic separation and time–of–flight mass spectrometry (TOFMS).

Mancozeb lead to *m/z* 144 ion fragmentation, which is 5–Imidazoledithiocarboxylic acid, as a major degradation product. ETU showed M^+ 102 which corresponds to its mass, was stable in distilled water and did not undergo hydrolysis during 60 minutes. The average retention time of mancozeb and ETU was approximately 181–189 and 210–230 seconds, respectively. Ozonation of mancozeb produced ETU as a major product. Treatment of ETU with ozone produced several degradation compounds. From prolonged ozonation, the CS_2 or CS group

was removed. Overall, several by-products identified were M^+ 60, M^+ 84, M^+ 163, M^+ 117 and M^+ 267 by ozone and M^+ 117, M^+ 86 and M^+ 163 by chlorine dioxide treatment. Several of these have been reported but some of those never been reported previously. Identification of fragment ions in this study was not conducted for unknown compounds but confirmed by comparison of published structural data with those of Degradation of Pesticides (1991). Although mancozeb and ETU were degraded by chlorine dioxide, this oxidant was less effective than ozone at the concentration used in this study. However, it is anticipated that mancozeb and ETU would be completely degraded by the chlorine dioxide treatment if the concentration of chlorine dioxide is increased above the 20 ppm that was used in this study.

FUTURE WORK

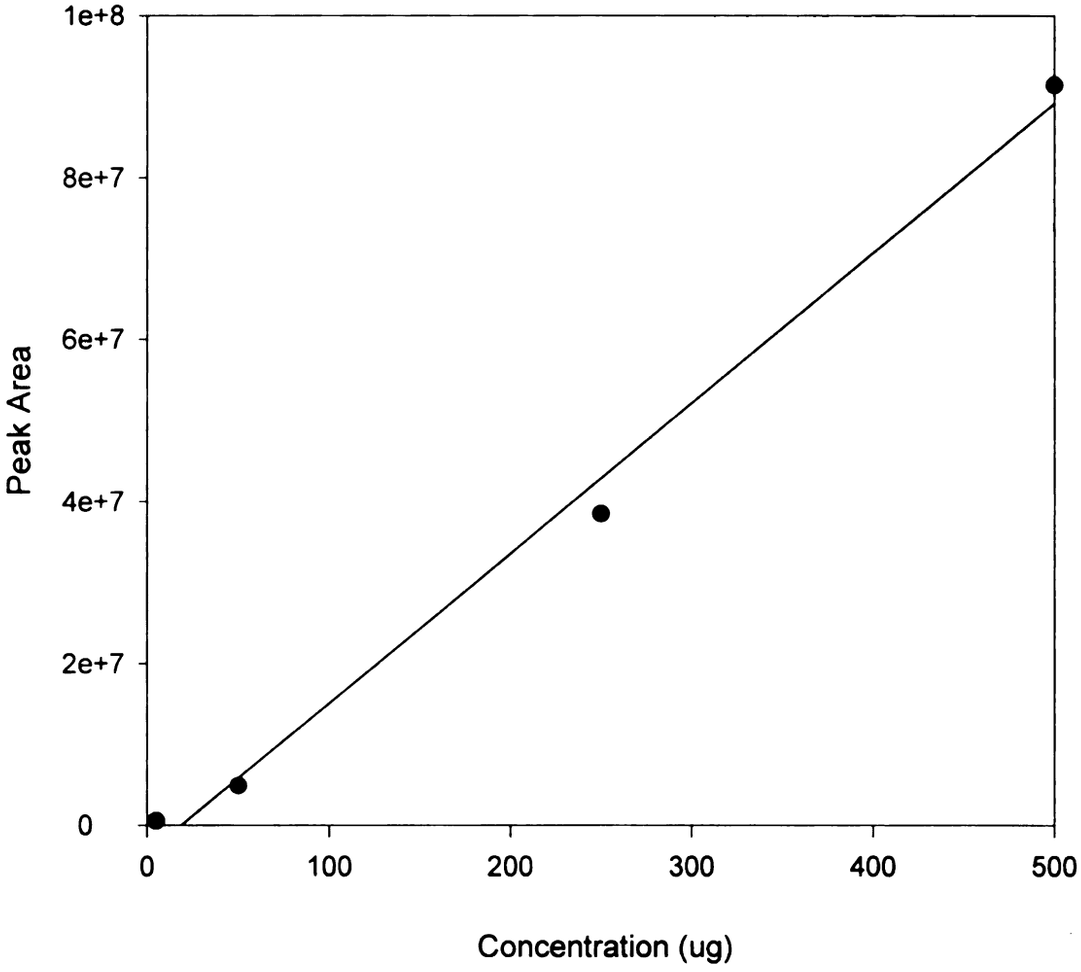
Possible future research efforts include:

1. This study determined that the various wash treatments and processing methods were effective in the degradation/removal of pesticide residues on apples at the pilot plant level. Future work should focus on the possibility of scaling up to a commercial size operation. More research should be carried out to set up the proper concentrations which may be used to maximized reductions of pesticide residue levels.

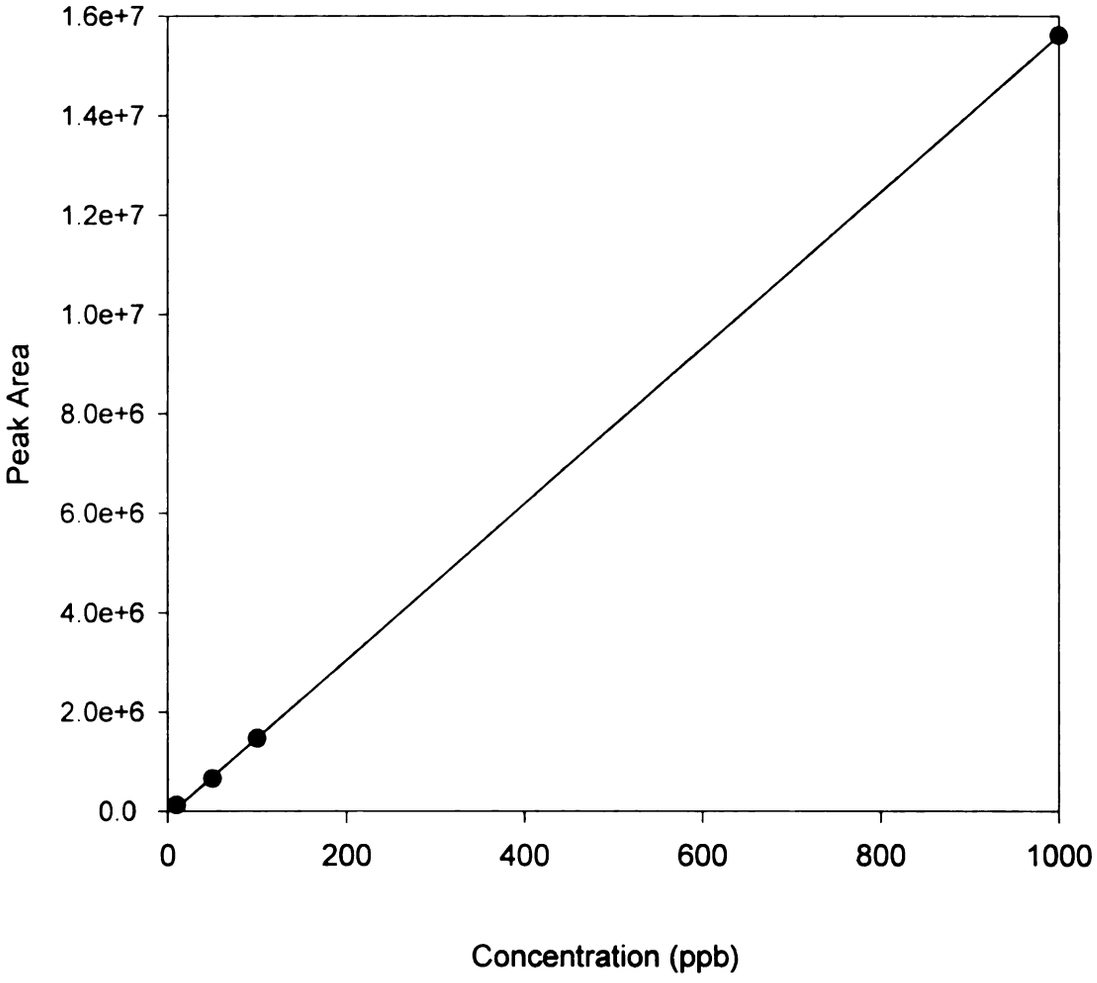
2. This study elucidated some degradation products and pathways after ozone and chlorine dioxide treatments in a model system. Future work should include determination of possible products as a results of chemical oxidation in processed apple products. Other analytical equipment, such as GC/MS, LC/MS, IR, NMR and UV, should be used to confirm the structure and pathways. Assessment of toxicity should also be carried out on the degradation products.

APPENDICES

Appendix 1. A typical standard curve for Mancozeb standards.



Appendix 2. A typical standard curve for ETU standards.



Appendix 3. Raw Data for Mancozeb Residues (ppm) with 2 ppm Spiked Mancozeb in a Model System.

Control @ pH 4.6

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	1.99	1.77	1.73	1.59	2.00	1.62	1.59	1.58
# 2	1.94	1.92	1.82	1.69	1.93	1.71	1.66	1.64
# 3	2.00	1.79	1.62	1.60	1.92	1.63	1.62	1.60
Ave.	1.98	1.83	1.72	1.63	1.95	1.65	1.62	1.61
Std.	0.03	0.08	0.10	0.06	0.04	0.05	0.04	0.03

Control @ pH 7.0

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	2.00	1.99	1.87	1.95	2.00	1.96	1.87	1.97
# 2	2.00	2.06	2.05	2.02	1.95	2.11	2.05	1.96
# 3	1.94	1.92	1.88	1.78	2.00	1.84	1.88	1.78
Ave.	1.98	1.99	1.93	1.92	1.98	1.97	1.93	1.90
Std.	0.03	0.07	0.10	0.12	0.03	0.14	0.10	0.11

Control @ pH 10.7

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	2.00	1.78	1.73	1.78	1.97	1.66	1.64	1.62
# 2	1.97	1.81	1.80	1.72	1.95	1.75	1.66	1.64
# 3	1.94	1.74	1.75	1.69	1.99	1.67	1.62	1.53
Ave.	1.97	1.78	1.76	1.73	1.97	1.69	1.64	1.60
Std.	0.03	0.04	0.04	0.05	0.02	0.05	0.02	0.06

Appendix 3. **(Cont'd)**

50 ppm $Ca(OCl)_2$ @ pH 4.6

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	1.99	0.27	0.27	0.17	2.00	N. D.	N. D.	N. D.
# 2	1.94	0.12	0.10	0.08	1.93	N. D.	N. D.	N. D.
# 3	2.00	0.14	0.11	0.10	1.92	N. D.	N. D.	N. D.
Ave.	1.98	0.18	0.16	0.12	1.95			
Std.	0.03	0.08	0.10	0.05	0.04			

50 ppm $Ca(OCl)_2$ @ pH 7.0

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	2.00	1.58	1.15	0.46	2.00	1.04	0.42	0.34
# 2	2.00	1.69	0.92	0.58	1.95	1.10	0.60	0.42
# 3	1.94	1.21	0.66	0.58	2.00	1.43	0.65	0.44
Ave.	1.98	1.49	0.91	0.54	1.98	1.19	0.56	0.40
Std.	0.03	0.25	0.25	0.07	0.03	0.21	0.12	0.05

50 ppm $Ca(OCl)_2$ @ pH 10.7

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	2.00	1.75	1.56	1.49	1.97	1.62	1.53	1.37
# 2	1.97	1.67	1.58	1.48	1.95	1.66	1.44	1.34
# 3	1.94	1.59	1.56	1.43	1.99	1.52	1.41	1.14
Ave.	1.97	1.67	1.57	1.47	1.97	1.60	1.46	1.28
Std.	0.03	0.08	0.01	0.03	0.02	0.07	0.06	0.13

Note: N. D. = None Detected. This represents a value <0.01ug/g, which is the method detection limit for mancozeb.

Appendix 3. **(Cont'd)**

250 ppm $Ca(OCl)_2$ @ pH 4.6

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	1.99	0.20	0.02	N. D.	2.00	N. D.	N. D.	N. D.
# 2	1.94	N. D.	N. D.	N. D.	1.93	N. D.	N. D.	N. D.
# 3	2.00	0.02	N. D.	N. D.	1.92	N. D.	N. D.	N. D.
Ave.	1.98	0.11	0.02		1.95			
Std.	0.03	0.13			0.04			

250 ppm $Ca(OCl)_2$ @ pH 7.0

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	2.00	0.77	0.17	0.13	2.00	0.31	0.23	0.06
# 2	2.00	0.63	0.23	0.12	1.95	0.37	0.14	0.04
# 3	1.94	0.61	0.19	0.13	2.00	0.44	0.15	0.03
Ave.	1.98	0.67	0.20	0.13	1.98	0.37	0.17	0.04
Std.	0.03	0.09	0.03	0.01	0.03	0.07	0.05	0.02

250 ppm $Ca(OCl)_2$ @ pH 10.7

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	2.00	1.20	1.03	0.75	1.97	1.05	0.86	0.73
# 2	1.97	1.15	0.97	0.74	1.95	1.01	0.90	0.68
# 3	1.94	1.27	1.08	0.82	1.99	1.03	0.98	0.61
Ave.	1.97	1.21	1.03	0.77	1.97	1.03	0.91	0.67
Std.	0.03	0.06	0.06	0.04	0.02	0.02	0.06	0.06

Note: N. D. = None Detected. This represents a value <0.01ug/g, which is the method detection limit for mancozeb.

Appendix 3. **(Cont'd)**

500 ppm $Ca(OCl)_2$ @ pH 4.6

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	1.99	N. D.	N. D.	N. D.	2.00	N. D.	N. D.	N. D.
# 2	1.94	N. D.	N. D.	N. D.	1.93	N. D.	N. D.	N. D.
# 3	2.00	N. D.	N. D.	N. D.	1.92	N. D.	N. D.	N. D.
Ave.	1.98				1.95			
Std.	0.03				0.04			

500 ppm $Ca(OCl)_2$ @ pH 7.0

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	2.00	0.44	0.07	0.06	2.00	0.15	0.09	N. D.
# 2	2.00	0.12	0.11	0.07	1.95	0.26	0.03	N. D.
# 3	1.94	0.22	0.11	0.07	2.00	0.24	N. D.	N. D.
Ave.	1.98	0.26	0.10	0.07	1.98	0.22	0.06	
Std.	0.03	0.16	0.02	0.01	0.03	0.06	0.04	

500 ppm $Ca(OCl)_2$ @ pH 10.7

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	2.00	0.62	0.17	0.15	1.97	0.60	0.49	0.15
# 2	1.97	0.68	0.42	0.26	1.95	0.58	0.41	0.26
# 3	1.94	0.73	0.39	0.36	1.99	0.49	0.39	0.29
Ave.	1.97	0.68	0.33	0.26	1.97	0.56	0.43	0.23
Std.	0.03	0.06	0.14	0.11	0.02	0.06	0.05	0.07

Note: N. D. = None Detected. This represents a value <0.01ug/g, which is the method detection limit for mancozeb.

Appendix 3. **(Cont'd)**

5 ppm ClO_2 @ pH 4.6

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	1.99	0.64	0.62	0.55	2.00	0.55	0.40	0.39
# 2	1.94	0.81	0.64	0.58	1.93	0.54	0.46	0.45
# 3	2.00	0.78	0.69	0.70	1.92	0.62	0.48	0.44
Ave.	1.98	0.74	0.65	0.61	1.95	0.57	0.45	0.43
Std.	0.03	0.09	0.04	0.08	0.04	0.04	0.04	0.03

5 ppm ClO_2 pH 7.0

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	2.00	1.28	1.26	1.09	2.00	1.23	0.77	0.64
# 2	2.00	1.01	0.95	0.92	1.95	1.22	0.74	0.41
# 3	1.94	1.14	0.92	0.90	2.00	1.02	0.66	0.45
Ave.	1.98	1.14	1.04	0.97	1.98	1.16	0.72	0.50
Std.	0.03	0.14	0.19	0.10	0.03	0.12	0.06	0.12

5 ppm ClO_2 @ pH 10.7

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	2.00	1.46	1.18	1.05	1.97	0.93	0.88	0.91
# 2	1.97	1.66	1.41	1.37	1.95	1.12	1.12	1.03
# 3	1.94	1.86	1.75	1.40	1.99	1.25	1.19	0.89
Ave.	1.97	1.66	1.45	1.27	1.97	1.10	1.06	0.94
Std.	0.03	0.20	0.29	0.19	0.02	0.16	0.16	0.08

Appendix 3. **(Cont'd)**

10 ppm ClO_2 @ pH 4.6

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	1.99	0.23	0.31	0.16	2.00	0.06	0.05	0.03
# 2	1.94	0.22	0.21	0.18	1.93	0.06	0.05	0.06
# 3	2.00	0.43	0.17	0.42	1.92	0.19	0.10	0.09
Ave.	1.98	0.29	0.23	0.25	1.95	0.10	0.07	0.06
Std.	0.03	0.12	0.07	0.14	0.04	0.08	0.03	0.03

10 ppmClO_2 @ pH 7.0

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	2.00	1.38	1.09	0.91	2.00	1.22	0.80	0.57
# 2	2.00	1.02	0.73	0.49	1.95	1.15	0.52	0.31
# 3	1.94	0.96	0.70	0.67	2.00	0.81	0.56	0.33
Ave.	1.98	1.12	0.84	0.69	1.98	1.06	0.63	0.40
Std.	0.03	0.23	0.22	0.21	0.03	0.22	0.15	0.14

10 ppm ClO_2 @ pH 10.7

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	2.00	1.12	1.08	0.81	1.97	1.03	0.94	0.84
# 2	1.97	1.58	1.20	0.85	1.95	1.39	1.12	1.06
# 3	1.94	1.82	1.26	0.96	1.99	1.16	1.17	0.83
Ave.	1.97	1.51	1.18	0.87	1.97	1.19	1.08	0.91
Std.	0.03	0.36	0.09	0.08	0.02	0.18	0.12	0.13

Appendix 3. **(Cont'd)**

1 ppm O_3 @ pH 4.6

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	1.99	1.60	1.62	1.55	2.00	1.61	1.23	1.08
# 2	1.94	1.36	1.53	1.50	1.93	1.31	1.09	0.83
# 3	2.00	1.22	0.96	0.87	1.92	0.86	0.86	0.60
Ave.	1.98	1.39	1.37	1.31	1.95	1.26	1.06	0.84
Std.	0.03	0.19	0.36	0.38	0.04	0.38	0.19	0.24

1 ppm O_3 @ pH 7.0

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	2.00	0.76	0.71	0.50	2.00	0.60	0.46	0.05
# 2	2.00	0.70	0.58	0.54	1.95	0.59	0.32	0.07
# 3	1.94	0.95	0.61	0.58	2.00	1.14	0.57	0.12
Ave.	1.98	0.80	0.63	0.54	1.98	0.78	0.45	0.08
Std.	0.03	0.13	0.07	0.04	0.03	0.31	0.13	0.04

1 ppm O_3 @ pH 10.7

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	2.00	1.91	1.66	1.58	1.97	1.71	1.70	1.44
# 2	1.97	1.89	1.77	1.66	1.95	1.54	1.52	1.18
# 3	1.94	1.92	1.87	1.76	1.99	1.63	1.26	1.01
Ave.	1.97	1.91	1.77	1.67	1.97	1.63	1.49	1.21
Std.	0.03	0.02	0.11	0.09	0.02	0.09	0.22	0.22

Appendix 3. **(Cont'd)**

3 ppm O_3 @ pH 4.6

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	1.99	1.45	1.46	1.25	2.00	1.00	0.56	0.19
# 2	1.94	1.43	1.28	1.25	1.93	1.11	0.69	0.36
# 3	2.00	1.01	0.92	0.91	1.92	0.84	0.55	0.36
Ave.	1.98	1.30	1.22	1.14	1.95	0.98	0.60	0.30
Std.	0.03	0.25	0.27	0.20	0.04	0.14	0.08	0.10

3 ppm O_3 @ pH 7.0

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	2.00	0.98	0.79	0.87	2.00	0.44	0.14	0.05
# 2	2.00	0.49	0.33	0.29	1.95	0.15	0.14	N. D.
# 3	1.94	0.43	0.29	0.29	2.00	0.21	0.12	N. D.
Ave.	1.98	0.63	0.47	0.48	1.98	0.27	0.13	0.05
Std.	0.03	0.30	0.28	0.33	0.03	0.15	0.01	

3 ppm O_3 @ pH 10.7

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	2.00	1.73	1.65	1.63	1.97	1.36	1.37	1.15
# 2	1.97	1.49	1.40	1.51	1.95	1.29	1.25	1.13
# 3	1.94	1.51	1.38	1.33	1.99	1.33	1.34	1.12
Ave.	1.97	1.58	1.48	1.49	1.97	1.33	1.32	1.13
Std.	0.03	0.13	0.15	0.15	0.02	0.04	0.06	0.02

Note: N. D. = None Detected. This represents a value <0.01ug/g, which is the method detection limit for mancozeb.

Appendix 3. **(Cont'd)**

5 ppm HPAA @ pH 4.6

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	1.99	1.48	1.47	1.37	2.00	1.39	1.06	0.94
# 2	1.94	1.72	1.33	1.31	1.93	1.26	0.89	0.71
# 3	2.00	1.99	1.84	1.74	1.92	1.42	1.22	0.95
Ave.	1.98	1.73	1.55	1.47	1.95	1.36	1.06	0.87
Std.	0.03	0.26	0.26	0.23	0.04	0.09	0.17	0.14

5 ppm HPAA @ pH 7.0

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	2.00	1.87	0.77	0.39	2.00	1.31	0.45	0.20
# 2	2.00	1.20	0.51	0.34	1.95	0.91	0.39	0.25
# 3	1.94	1.23	0.63	0.41	2.00	0.79	0.26	0.06
Ave.	1.98	1.43	0.64	0.38	1.98	1.00	0.37	0.17
Std.	0.03	0.38	0.13	0.04	0.03	0.27	0.10	0.10

5 ppm HPAA @ pH 10.7

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	2.00	1.61	1.29	1.16	1.97	1.13	1.05	0.78
# 2	1.97	1.49	1.46	1.09	1.95	1.18	1.03	0.64
# 3	1.94	1.60	1.36	1.28	1.99	1.25	1.26	0.81
Ave.	1.97	1.57	1.37	1.18	1.97	1.19	1.11	0.74
Std.	0.03	0.07	0.09	0.10	0.02	0.06	0.13	0.09

Appendix 3. **(Cont'd)**

50 ppm HPAA @ pH 4.6

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	1.99	1.57	1.28	0.95	2.00	0.71	0.28	0.22
# 2	1.94	1.53	1.27	0.87	1.93	1.11	0.62	0.34
# 3	2.00	1.74	1.20	0.99	1.92	1.18	0.65	0.38
Ave.	1.98	1.61	1.25	0.94	1.95	1.00	0.52	0.31
Std.	0.03	0.11	0.04	0.06	0.04	0.25	0.21	0.08

50 ppm HPAA @ pH 7.0

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	2.00	0.12	0.03	0.03	2.00	0.06	N. D.	N. D.
# 2	2.00	0.15	0.03	N. D.	1.95	0.04	N. D.	N. D.
# 3	1.94	0.09	0.03	N. D.	2.00	0.08	N. D.	N. D.
Ave.	1.98	0.12	0.03	0.03	1.98	0.06		
Std.	0.03	0.03	0.00		0.03	0.02		

50 ppm HPAA @ pH 10.7

	10 C				21 C			
	0 min	5 min	15 min	30 min	0 min	5 min	15 min	30 min
# 1	2.00	0.64	0.08	N. D.	1.97	0.41	N. D.	N. D.
# 2	1.97	0.74	0.10	N. D.	1.95	1.02	N. D.	N. D.
# 3	1.94	0.72	0.14	N. D.	1.99	0.33	N. D.	N. D.
Ave.	1.97	0.70	0.11		1.97	0.59		
Std.	0.03	0.05	0.03		0.02	0.38		

Note: N. D. = None Detected. This represents a value <0.01ug/g, which is the method detection limit for mancozeb.

Appendix 4. Raw data for ETU residues (ppb) with 2 ppm spiked Mancozeb in a model system.

	Control @ pH 4.6					Control @ pH 7.0				
	0 min	5 min	15 min	30 min	60 min	0 min	5 min	15 min	30 min	60 min
# 1	10.90	8.19	13.03	6.18	5.93	15.42	20.41	20.83	20.87	11.08
# 2	11.05	14.21	12.38	8.79	5.06	17.25	17.69	19.76	16.17	15.52
# 3	13.60	16.30	17.34	7.99	4.76	19.25	20.45	25.11	20.46	10.30
Ave.	11.85	12.90	14.25	7.65	5.25	17.31	19.52	21.90	19.17	12.30
Std.	1.52	4.21	2.70	1.34	0.61	1.92	1.58	2.83	2.60	2.82

	Control @ pH 10.7				
	0 min	5 min	15 min	30 min	60 min
# 1	16.34	15.18	20.98	16.92	10.95
# 2	13.50	14.71	18.27	15.76	10.37
# 3	15.17	16.46	17.90	16.37	14.69
Ave.	15.00	15.45	19.05	16.35	12.00
Std.	1.43	0.91	1.68	0.58	2.34

Appendix 4. (Cont'd)

	50 ppm $Ca(OCl)_2$ @ pH 4.6					250 ppm$Ca(OCl)_2$ @ pH 4.6				
	0 min	5 min	15 min	30 min	60 min	0 min	5 min	15 min	30 min	60 min
# 1	10.90	N. D.	N. D.	N. D.	N. D.	10.90	N. D.	N. D.	N. D.	N. D.
# 2	11.05	N. D.	N. D.	N. D.	N. D.	11.05	N. D.	N. D.	N. D.	N. D.
# 3	13.60	N. D.	N. D.	N. D.	N. D.	13.60	N. D.	N. D.	N. D.	N. D.
Ave.	11.85					11.85				
Std.	1.52					1.52				

	50 ppm $Ca(OCl)_2$ @ pH 7.0					250 ppm $Ca(OCl)_2$ @ pH 7.0				
	0 min	5 min	15 min	30 min	60 min	0 min	5 min	15 min	30 min	60 min
# 1	15.42	9.52	6.15	5.67	N. D.	15.42	N. D.	N. D.	N. D.	N. D.
# 2	17.25	13.49	7.23	7.08	N. D.	17.25	N. D.	N. D.	N. D.	N. D.
# 3	19.25	10.74	8.22	7.50	N. D.	19.25	N. D.	N. D.	N. D.	N. D.
Ave.	17.31	11.25	7.20	6.75		17.31				
Std.	1.92	2.03	1.04	0.96		1.92				

	50 ppm $Ca(OCl)_2$ @ pH 10.7					250 ppm $Ca(OCl)_2$ @ pH 10.7				
	0 min	5 min	15 min	30 min	60 min	0 min	5 min	15 min	30 min	60 min
# 1	16.34	15.56	12.13	8.67	4.52	16.34	10.43	9.12	8.69	7.70
# 2	13.50	13.49	11.29	10.08	6.31	13.50	8.79	9.20	9.25	7.23
# 3	15.17	11.01	10.33	10.50	4.91	15.17	9.59	8.22	8.15	8.03
Ave.	15.00	13.35	11.25	9.75	5.25	15.00	9.60	8.85	8.70	7.65
Std.	1.43	2.28	0.90	0.96	0.94	1.43	0.82	0.54	0.55	0.40

Note: N. D. = None detected. This represents a value <5ng/g which is the method of detection limit for ETU in solutions.

Appendix 4. (Cont'd)

	5 ppm ClO_2 @ pH 4.6					10 ppm ClO_2 @ pH 4.6				
	0 min	5 min	15 min	30 min	60 min	0 min	5 min	15 min	30 min	60 min
# 1	10.90	N. D.	N. D.	N. D.	N. D.	10.90	N. D.	N. D.	N. D.	N. D.
# 2	11.05	N. D.	N. D.	N. D.	N. D.	11.05	N. D.	N. D.	N. D.	N. D.
# 3	13.60	N. D.	N. D.	N. D.	N. D.	13.60	N. D.	N. D.	N. D.	N. D.
Ave.	11.85					11.85				
Std.	1.52					1.52				

	5 ppm ClO_2 @ pH 7.0					10 ppm ClO_2 @ pH 7.0				
		5 min	15 min	30 min	60 min	0 min	5 min	15 min	30 min	60 min
# 1	15.42	10.34	6.13	5.73	4.52	15.42	5.03	4.77	2.92	N. D.
# 2	17.25	9.16	7.49	6.09	5.87	17.25	4.85	3.76	N. D	N. D.
# 3	19.25	8.86	7.07	6.18	4.91	19.25	5.42	4.52	2.48	N. D.
Ave.	17.31	9.45	6.90	6.00	5.10	17.31	5.10	4.35	2.70	
Std.	1.92	0.78	0.70	0.24	0.69	1.92	0.29	0.53	0.31	

	5 ppm ClO_2 @ pH 10.7					10 ppm ClO_2 @ pH 10.7				
	0 min	5 min	15 min	30 min	60 min	0 min	5 min	15 min	30 min	60 min
# 1	16.34	9.56	8.96	7.34	4.89	16.34	10.43	5.32	3.69	N. D.
# 2	13.50	10.04	10.01	8.12	6.39	13.50	9.24	4.84	3.54	N. D.
# 3	15.17	9.66	8.94	7.05	5.73	15.17	9.59	6.03	4.01	N. D.
Ave.	15.00	9.75	9.30	7.50	5.67	15.00	9.75	5.40	3.75	
Std.	1.43	0.25	0.61	0.55	0.75	1.43	0.61	0.60	0.24	

Note: N. D. = None detected. This represents a value <5ng/g which is the method of detection limit for ETU in solutions.

Appendix 4. (Cont'd)

	1 ppm O_3 @ pH 4.6					3 ppm O_3 @ pH 4.6				
	0 min	5 min	15 min	30 min	60 min	0 min	5 min	15 min	30 min	60 min
# 1	10.90	7.54	6.32	N. D.	N. D.	10.90	N. D.	N. D.	N. D.	N. D.
# 2	11.05	8.92	7.73	N. D.	N. D.	11.05	N. D.	N. D.	N. D.	N. D.
# 3	13.60	9.20	8.46	N. D.	N. D.	13.60	N. D.	N. D.	N. D.	N. D.
Ave.	11.85	8.55	7.50			11.85				
Std.	1.52	0.89	1.09			1.52				

	1 ppm O_3 @ pH 7.0					3 ppm O_3 @ pH 7.0				
	0 min	5 min	15 min	30 min	60 min	0 min	5 min	15 min	30 min	60 min
# 1	15.42	11.79	5.29	N. D.	N. D.	15.42	N. D.	N. D.	N. D.	N. D.
# 2	17.25	12.87	6.17	N. D.	N. D.	17.25	N. D.	N. D.	N. D.	N. D.
# 3	19.25	10.43	4.73	N. D.	N. D.	19.25	N. D.	N. D.	N. D.	N. D.
Ave.	17.31	11.70	5.40			17.31				
Std.	1.92	1.22	0.73			1.92				

	1 ppm O_3 @ pH 10.7					3 ppm O_3 @ pH 10.7				
	0 min	5 min	15 min	30 min	60 min	0 min	5 min	15 min	30 min	60 min
# 1	16.34	10.01	9.75	8.48	8.02	16.34	4.38	N. D.	N. D.	N. D.
# 2	13.50	9.23	9.11	8.71	7.37	13.50	4,75	N. D.	N. D.	N. D.
# 3	15.17	8.65	8.12	7.57	6.67	15.17	5.81	N. D.	N. D.	N. D.
Ave.	15.00	9.30	8.99	8.25	7.35	15.00	5.10			
Std.	1.43	0.68	0.82	0.60	0.68	1.43	1.01			

Note: N. D. = None detected. This represents a value <5ng/g which is the method of detection limit for ETU in solutions.

Appendix 4. **(Cont'd)**

	5 ppm HPAA @ pH 4.6					50 ppm HPAA @ pH 4.6				
	0 min	5 min	15 min	30 min	60 min	0 min	5 min	15 min	30 min	60 min
# 1	10.90	N. D.	N. D.	N. D.	N. D.	10.90	N. D.	N. D.	N. D.	N. D.
# 2	11.05	N. D.	N. D.	N. D.	N. D.	11.05	N. D.	N. D.	N. D.	N. D.
# 3	13.60	N. D.	N. D.	N. D.	N. D.	13.60	N. D.	N. D.	N. D.	N. D.
Ave.	11.85					11.85				
Std.	1.52					1.52				

	5 ppm HPAA @ pH 7.0					50 ppm HPAA @ pH 7.0				
	0 min	5 min	15 min	30 min	60 min	0 min	5 min	15 min	30 min	60 min
# 1	15.42	7.85	6.84	4.32	N. D.	15.42	7.03	4.54	N. D.	N. D.
# 2	17.25	8.09	7.31	6.02	N. D.	17.25	6.44	5.88	N. D.	N. D.
# 3	19.25	8.37	7.89	5.40	N. D.	19.25	5.87	5.77	N. D.	N. D.
Ave.	17.31	8.10	7.35	5.25		17.31	6.45	5.40		
Std.	1.92	0.26	0.53	0.86		1.92	0.58	0.74		

	5 ppm HPAA @ pH 10.7					50 ppm HPAA @ pH 10.7				
	0 min	5 min	15 min	30 min	60 min	0 min	5 min	15 min	30 min	60 min
# 1	16.34	N. D.	N. D	N. D.	N. D	16.34	N. D.	N. D.	N. D.	N. D.
# 2	13.50	N. D.	N. D	N. D.	N. D	13.50	N. D.	N. D.	N. D.	N. D.
# 3	15.17	N. D.	N. D	N. D.	N. D	15.17	N. D.	N. D.	N. D.	N. D.
Ave.	15.00					15.00				
Std.	1.43					1.43				

Note: N. D. = None detected. This represents a value <5ng/g which is the method of detection limit for ETU in solutions.

Appendix 5. Raw data for Mancozeb residues (ppm) in 1 and 10 ppm Mancozeb spiked apples.

Control

	0 min		5 min		15 min		30 min	
	Spiked Mancozeb		Spiked Mancozeb		Spiked Mancozeb		Spiked Mancozeb	
	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm
# 1	1.00	9.85	0.96	9.01	0.90	8.90	0.81	8.53
# 2	1.03	10.54	0.89	9.99	0.85	10.19	0.82	10.11
# 3	1.00	10.19	0.94	9.67	0.88	9.55	0.85	9.32
Ave.	1.01	10.19	0.93	9.56	0.88	9.55	0.83	9.32
Std.	0.02	0.35	0.04	0.50	0.03	0.65	0.02	0.79

50 ppm $Ca(OCl)_2$

	0 min		5 min		15 min		30 min	
	Spiked Mancozeb		Spiked Mancozeb		Spiked Mancozeb		Spiked Mancozeb	
	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm
# 1	1.00	9.85	0.52	3.85	0.27	3.76	0.08	3.00
# 2	1.03	10.54	0.30	3.97	0.34	1.95	0.14	1.66
# 3	1.00	10.19	0.41	3.91	0.30	2.86	0.11	2.33
Ave.	1.01	10.19	0.41	3.91	0.30	2.86	0.11	2.33
Std.	0.02	0.35	0.11	0.06	0.04	0.91	0.03	0.67

500 ppm $Ca(OCl)_2$

	0 min		5 min		15 min		30 min	
	Spiked Mancozeb		Spiked Mancozeb		Spiked Mancozeb		Spiked Mancozeb	
	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm
# 1	1.00	9.85	0.08	2.21	0.01	0.91	0.01	0.57
# 2	1.03	10.54	0.05	2.58	0.01	0.92	0.01	0.58
# 3	1.00	10.19	0.06	2.40	0.01	0.92	0.01	0.58
Ave.	1.01	10.19	0.06	2.40	0.01	0.92	0.01	0.58
Std.	0.02	0.35	0.02	0.19	0.00	0.01	0.00	0.01

Appendix 5. (Cont'd)

5 ppm ClO_2

	0 min		5 min		15 min		30 min	
	Spiked Mancozeb		Spiked Mancozeb		Spiked Mancozeb		Spiked Mancozeb	
	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm
# 1	1.00	9.85	0.37	5.64	0.27	5.02	0.16	3.23
# 2	1.03	10.54	0.28	6.60	0.24	4.42	0.24	4.33
# 3	1.00	10.19	0.32	6.12	0.25	4.72	0.20	3.78
Ave.	1.01	10.19	0.32	6.12	0.25	4.72	0.20	3.78
Std.	0.02	0.35	0.05	0.48	0.02	0.30	0.04	0.55

10 ppm ClO_2

	0 min		5 min		15 min		30 min	
	Spiked Mancozeb		Spiked Mancozeb		Spiked Mancozeb		Spiked Mancozeb	
	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm
# 1	1.00	9.85	0.26	1.52	0.20	1.40	0.17	1.09
# 2	1.03	10.54	0.35	1.59	0.26	1.41	0.19	1.24
# 3	1.00	10.19	0.30	1.56	0.23	1.41	0.18	1.17
Ave.	1.01	10.19	0.30	1.56	0.23	1.41	0.18	1.17
Std.	0.02	0.35	0.05	0.04	0.03	0.01	0.01	0.08

1 ppm O_3

	0 min		5 min		15 min		30 min	
	Spiked Mancozeb		Spiked Mancozeb		Spiked Mancozeb		Spiked Mancozeb	
	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm
# 1	1.00	9.85	0.43	3.58	0.27	3.16	0.15	2.23
# 2	1.03	10.54	0.39	3.20	0.36	2.91	0.22	2.62
# 3	1.00	10.19	0.41	3.39	0.32	3.03	0.19	2.43
Ave.	1.01	10.19	0.41	3.39	0.32	3.03	0.19	2.43
Std.	0.02	0.35	0.02	0.19	0.05	0.13	0.04	0.20

Appendix 5. (Cont'd)

3 ppm O_3

	0 min		5 min		15 min		30 min	
	Spiked Mancozeb		Spiked Mancozeb		Spiked Mancozeb		Spiked Mancozeb	
	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm
# 1	1.00	9.85	0.30	3.97	0.23	2.54	0.12	0.26
# 2	1.03	10.54	0.37	4.09	0.20	2.73	0.14	0.36
# 3	1.00	10.19	0.34	4.03	0.21	2.64	0.13	0.31
Ave.	1.01	10.19	0.34	4.03	0.21	2.64	0.13	0.31
Std.	0.02	0.35	0.04	0.06	0.02	0.10	0.01	0.05

50 ppm HPAA

	0 min		5 min		15 min		30 min	
	Spiked Mancozeb		Spiked Mancozeb		Spiked Mancozeb		Spiked Mancozeb	
	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm
# 1	1.00	9.85	0.33	5.00	0.27	3.97	0.17	3.23
# 2	1.03	10.54	0.58	5.75	0.31	4.81	0.11	3.03
# 3	1.00	10.19	0.57	5.37	0.44	4.39	0.14	3.13
Ave.	1.01	10.19	0.49	5.37	0.34	4.39	0.14	3.13
Std.	0.02	0.35	0.14	0.38	0.09	0.42	0.03	0.10

500 ppm HPAA

	0 min		5 min		15 min		30 min	
	Spiked Mancozeb		Spiked Mancozeb		Spiked Mancozeb		Spiked Mancozeb	
	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm	1 ppm	10 ppm
# 1	1.00	9.85	0.24	2.99	0.10	0.63	0.01	0.11
# 2	1.03	10.54	0.24	3.43	0.08	0.83	0.01	0.15
# 3	1.00	10.19	0.24	3.21	0.09	0.73	0.01	0.13
Ave.	1.01	10.19	0.24	3.21	0.09	0.73	0.01	0.13
Std.	0.02	0.35	0.00	0.22	0.01	0.10	0.00	0.02

Appendix 6. Raw Data for Mancozeb Residues (ppm) in Apples and Apple Products (1997)

	Whloe Fruit				Slices			
	1	2	3	4	1	2	3	4
# 1	0.14	0.04	N. D.	N. D.	N. D	N. D.	N. D	N. D
# 2	0.05	N. D.	N. D.	N. D.	N. D	N. D.	N. D	N. D
# 3	0.08	N. D.	N. D.	N. D.	N. D	N. D.	N. D	N. D
Ave.	0.09	0.04						
Std.	0.05							

	Unpeeled Sauce				Peeled Sauce			
	1	2	3	4	1	2	3	4
# 1	N. D	N. D.	N. D	N. D	N. D	N. D.	N. D	N. D
# 2	N. D	N. D.	N. D	N. D	N. D	N. D.	N. D	N. D
# 3	N. D	N. D.	N. D	N. D	N. D	N. D.	N. D	N. D
Ave.								
Std.								

	Juice				Pomace			
	1	2	3	4	1	2	3	4
# 1	0.08	0.06	N. D	N. D.	0.13	0.04	0.04	N. D
# 2	0.05	0.04	N. D	N. D.	0.08	0.04	0.03	N. D
# 3	0.09	0.07	N. D	N. D.	0.09	0.07	0.05	N. D
Ave.	0.07	0.06			0.10	0.05	0.04	
Std.	0.02	0.02			0.03	0.02	0.01	

* Note: N.D. = None detected.

This represents a value <0.01ug/g, which is the method detection limit for apple products.

* Wash treatments: 1. No wash 2. Water wash 3. 50 ppm $Ca(OCl)_2$ wash 4. 500 ppm $Ca(OCl)_2$ wash

Appendix 7. Raw Data for ETU Residues (ppm) in Apples and Apple Products (1997)

	Whloe Fruit				Slices			
	1	2	3	4	1	2	3	4
# 1	N. D	N. D.	N. D	N. D	N. D	N. D.	N. D	N. D
# 2	N. D	N. D.	N. D	N. D	N. D	N. D.	N. D	N. D
# 3	N. D	N. D.	N. D	N. D	N. D	N. D.	N. D	N. D
Ave.								
Std.								

	Unpeeled Sauce				Peeled Sauce			
	1	2	3	4	1	2	3	4
# 1	N. D	N. D.	N. D	N. D	N. D	N. D.	N. D	N. D
# 2	N. D	N. D.	N. D	N. D	N. D	N. D.	N. D	N. D
# 3	N. D	N. D.	N. D	N. D	N. D	N. D.	N. D	N. D
Ave.								
Std.								

	Juice				Pomace			
	1	2	3	4	1	2	3	4
# 1	N. D	N. D.	N. D	N. D.	0.05	N. D.	N. D	N. D
# 2	N. D	N. D.	N. D	N. D.	0.07	N. D.	N. D	N. D
# 3	N. D	N. D.	N. D	N. D.	0.03	N. D.	N. D	N. D
Ave.					0.05			
Std.					0.02			

* Note: N.D. = None detected.
This represents a value <0.01ug/g, which is the method detection limit for apple products.
* Wash treatments: 1. No wash 2. Water wash 3. 50 ppm $Ca(OCl)_2$ wash 4. 500 ppm $Ca(OCl)_2$ wash

Appendix 8. Raw Data for Mancozeb Residues in Apples and Apple Products (1998)

Whole Fruit

	Mancozeb (ppm)						
	1	2	3	4	5	6	7
# 1	2.50	1.26	0.97	N. D.	0.65	N. D.	0.85
# 2	1.44	1.22	1.25	N. D.	0.80	N. D.	0.78
# 3	2.04	1.42	1.44	N. D.	0.72	N. D.	0.79
Ave.	1.99	1.30	1.22		0.72		0.81
Std.	0.53	0.11	0.24		0.08		0.04

Slices

	Mancozeb (ppm)						
	1	2	3	4	5	6	7
# 1	0.70	0.67	0.73	N. D.	N. D.	N. D.	N. D.
# 2	0.87	0.64	0.45	N. D.	N. D.	N. D.	N. D.
# 3	0.67	0.67	0.64	N. D.	N. D.	N. D.	N. D.
Ave.	0.75	0.66	0.61				
Std.	0.11	0.02	0.14				

* Note: N.D. = None detected.
This represents a value <0.01ug/g, which is the method detection limit for apple products.

* Wash treatments: 1. No wash 2. Water wash 3. 50 ppm $Ca(OCl)_2$ wash 4. 500 ppm $Ca(OCl)_2$ wash
5. 10 ppm ClO_2 wash 6. 3 ppm O_3 wash 7. 50 ppm HPAA wash

Appendix 8. **(Cont'd)**

Unpeeled Sauce

	Mancozeb (ppm)						
	1	2	3	4	5	6	7
# 1	0.60	0.57	N. D.	N. D.	N. D.	N. D.	N. D.
# 2	0.69	0.42	N. D.	N. D.	N. D.	N. D.	N. D.
# 3	0.64	0.58	N. D.	N. D.	N. D.	N. D.	N. D.
Ave.	0.64	0.52					
Std.	0.05	0.09					

Peeled Sauce

	Mancozeb (ppm)						
	1	2	3	4	5	6	7
# 1	0.58	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.
# 2	0.54	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.
# 3	0.45	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.
Ave.	0.52						
Std.	0.07						

* Note: N.D. = None detected.

This represents a value <0.01ug/g, which is the method detection limit for apple products.

* Wash treatments: 1. No wash 2. Water wash 3. 50 ppm $Ca(OCl)_2$ wash 4. 500 ppm $Ca(OCl)_2$ wash 5. 10 ppm ClO_2 wash 6. 3 ppm O_3 wash 7. 50 ppm HPAA wash

Appendix 8. **(Cont'd)**

Juice

	Mancozeb (ppm)						
	1	2	3	4	5	6	7
# 1	1.12	1.11	1.15	N. D.	0.80	0.68	0.71
# 2	1.34	0.78	0.86	N. D.	0.70	0.57	0.87
# 3	0.91	0.66	1.28	N. D.	0.68	0.71	0.68
Ave.	1.12	0.85	1.10		0.73	0.65	0.75
Std.	0.22	0.23	0.22		0.06	0.07	0.10

Pomace

	Mancozeb (ppm)						
	1	2	3	4	5	6	7
# 1	10.95	6.18	8.88	1.55	5.70	2.49	3.96
# 2	11.84	5.20	6.38	1.00	6.01	1.10	4.04
# 3	17.13	10.44	10.18	1.07	5.71	2.62	3.69
Ave.	13.31	7.27	8.48	1.21	5.81	2.07	3.90
Std.	3.34	2.79	1.93	0.30	0.18	0.84	0.18

* Note: N.D. = None detected.
This represents a value <0.01ug/g, which is the method detection limit for apple products.

* Wash treatments: 1. No wash 2. Water wash 3. 50 ppm $Ca(OCl)_2$ wash 4. 500 ppm $Ca(OCl)_2$ wash
5. 10 ppm ClO_2 wash 6. 3 ppm O_3 wash 7. 50 ppm HPAA wash

Appendix 9. Raw Data for ETU Residues in Apple and Apple Products (1998)

Whole Fruit

	ETU (ppb)						
	1	2	3	4	5	6	7
# 1	20.32	8.03	7.06	N. D.	N. D.	N. D.	N. D.
# 2	12.35	5.17	4.99	N. D.	N. D.	N. D.	N. D.
# 3	14.65	9.62	7.65	N. D.	N. D.	N. D.	N. D.
Ave.	15.77	7.61	6.57				
Std.	4.10	2.26	1.40				

Slices

	ETU (ppb)						
	1	2	3	4	5	6	7
# 1	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.
# 2	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.
# 3	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.
Ave.							
Std.							

* Note: N.D. = None detected.

This represents a value <0.01ug/g, which is the method detection limit for apple products.

* Wash treatments: 1. No wash 2. Water wash 3. 50 ppm $Ca(OCl)_2$ wash 4. 500 ppm $Ca(OCl)_2$ wash 5. 10 ppm ClO_2 wash 6. 3 ppm O_3 wash 7. 50 ppm HPAA wash

Appendix 9. (Cont'd)

Unpeeled Sauce

	ETU (ppb)						
	1	2	3	4	5	6	7
# 1	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.
# 2	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.
# 3	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.
Ave.							
Std.							

Peeled Sauce

	ETU (ppb)						
	1	2	3	4	5	6	7
# 1	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.
# 2	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.
# 3	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.
Ave.							
Std.							

* Note: N.D. = None detected.

This represents a value <0.01ug/g, which is the method detection limit for apple products.

* Wash treatments: 1. No wash 2. Water wash 3. 50 ppm $Ca(OCl)_2$ wash 4. 500 ppm $Ca(OCl)_2$ wash 5. 10 ppm ClO_2 wash 6. 3 ppm O_3 wash 7. 50 ppm HPAA wash

Appendix 9. (Cont'd)

Juice

	ETU (ppb)						
	1	2	3	4	5	6	7
# 1	6.18	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.
# 2	18.27	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.
# 3	5.21	N. D.	N. D.	N. D.	N. D.	N. D.	N. D.
Ave.	9.89						
Std.	7.28						

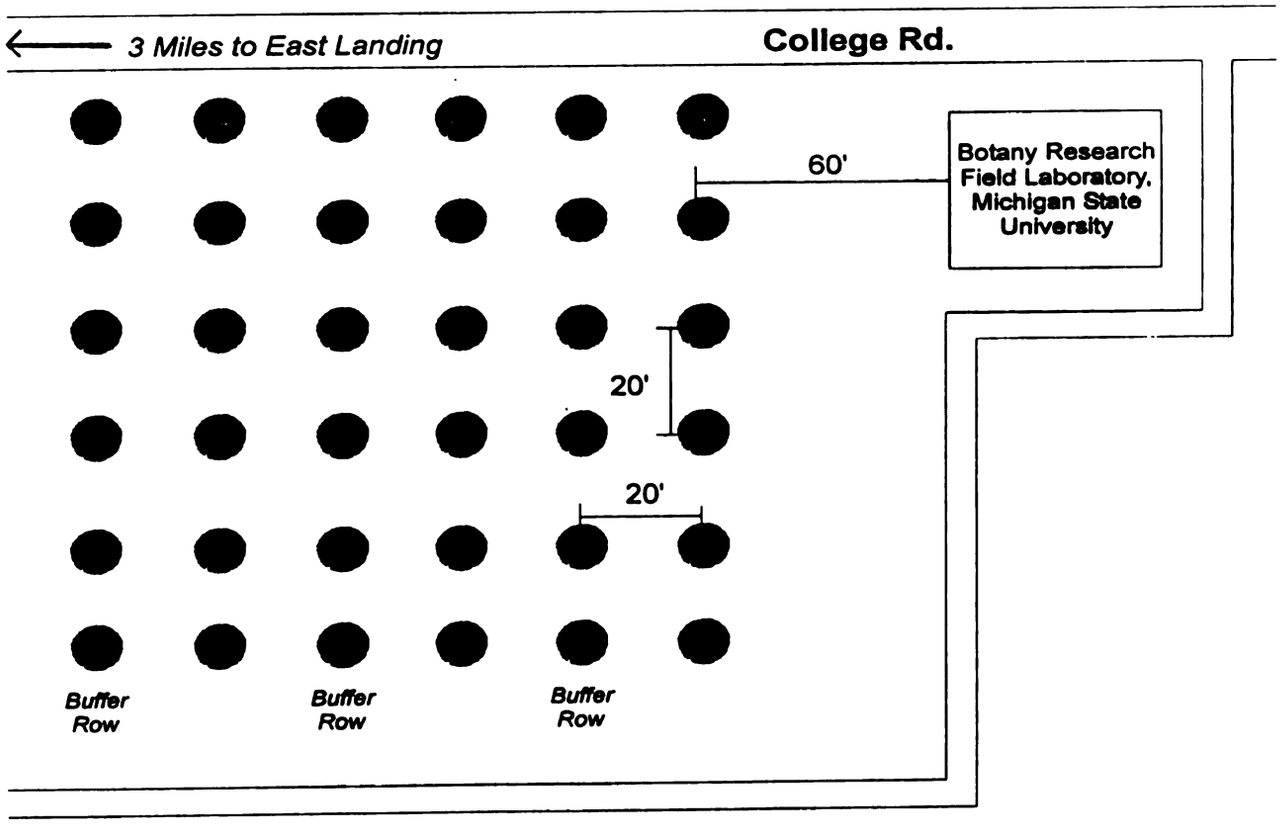
Pomace

	ETU (ppb)						
	1	2	3	4	5	6	7
# 1	74.79	46.87	34.58	16.06	25.16	13.19	16.98
# 2	92.60	59.61	45.66	12.57	28.73	9.49	20.89
# 3	50.05	42.62	40.51	11.33	23.37	12.75	16.60
Ave.	72.48	49.70	40.25	13.32	25.75	11.81	18.16
Std.	21.37	8.84	5.54	2.45	2.73	2.02	2.37

* Note: N.D. = None detected.
This represents a value <0.01ug/g, which is the method detection limit for apple products.

* Wash treatments: 1. No wash 2. Water wash 3. 50 ppm $Ca(OCl)_2$ wash 4. 500 ppm $Ca(OCl)_2$ wash
5. 10 ppm ClO_2 wash 6. 3 ppm O_3 wash 7. 50 ppm HPAA wash

Appendix 10. Field plot diagram



BIBLIOGRAPHY

BIBLIOGRAPHY

Ahmad, N., Guo, L., Mandarakas, P. and Appleby, S. 1995. Determination of dithiocarbamate and its breakdown product ethylenethiourea in fruits and vegetables. *J. Assoc. Off. Anal. Chem.* 78: 1238–1243.

Aieta, E. M. and Berg, J. D. 1986. A review of chlorine dioxide in drinking water treatment. *J. Am. Water Works Assoc.* 78: 62–70.

Aieta, E. M., Berg, J. D. and Roberts, P. V. 1980. Comparison of chlorine dioxide and chlorine in wastewater disinfection. *J. Water Pollut. Control Fed.*, 52: 810.

Aizawa, H. 1991. *Metabolic maps of pesticides.* Vol. 1. p. 54, Academic Press, Inc., San Diego, CA.

Ankumah, R. O. and Marshall, W. D. 1984. Persistence and fate of ethylenethiourea in tomato sauce and paste. *J. Agric. Food Chem.* 32: 1194–1198.

Arnold, D. L., Krewski, D. R., Junkins, D. B., McGuire, P. F., Moodie, C. A., and Munro, I. C. 1983. Reversibility of ethylenethiourea-induced thyroid lesions. *Toxicol. Appl. Pharmacol.* 67: 264–273.

Banrc (Board on Agriculture National Research Council). 1987. *Regulating pesticides in food.* National Academy Press, Washington, DC. 209.

Berg, G. L. 1988. *Farm Chemical Handbook.* Willoughby, OH: Meister Publishing Co.

Blazquez, C. H. 1973. Residue Determination of ethylenethiourea (2–imidazolidienthione) from tomato foliage, soil and water. *J. Agric. Food Chem.* 21: 330–332.

Block, S. B. 1991. Peroxygen compounds. In "*Disinfection, Sterilization, and Preservation*", 4th ed., p167. Lea and Febiger, Philadelphia.

Bohner, H. F. and Bradley, R. L. 1991. Corrosivity of chlorine dioxide used as sanitizer in ultrafiltration system. *J. Dairy Sci.* 74: 3348–3352.

Bontoyan, W. R., Looker, J. B., Kaiser, T. E., Giang, P. and Olive, B. M. 1972. Survey of ethylenethiourea in commercial ethylene bisdithiocarbamate formulations. *J. Assoc. Off. Anal. Chem.* 55: 923–925.

Bontoyan, W. R., Looker, J. B., Kaiser, T. E., Giang, P. and Olive, B. M. 1977. *J. Assoc. Off. Anal. Chem.* 60: 1105–1110.

Bottomley, P., Hoodless, R. A. and Smart, N. A. 1985. Review of methods for the determination of ethylenethiourea (imidazolidine-2-thione) residues. *Residue Reviews.* 95: 45–89.

Cabras, P., Meloni, M. and Pirisi, F. M. 1987. Pesticide fate from vine to wine. *Reviews Environ Contam Toxicol.* 99: 83–117.

Cash, J. N., Zabik, M. J. and Jones, A. L. 1997. The use of post harvest treatments and processing to reduce omite (propargite) residues in apples and apple products. Michigan State University. MI.

CFR Part 162.3. 1988. Littleton Colo.

Chernoff, N., Kavlock, R. J., Rogers, E. H., Carver, B. D. and Murray, S. 1979. Perinatal toxicity of maneb, ethylene thiourea, and ethylenebisthiocyanate sulfide in rodents. *J. Toxicol. Environ. Health.* 5: 821–834.

Clarke, D. G., Baum, H., Stanley, E. L. and Hester, W. F. 1951. Determination of dithiocarbamates. *Anal. Chem.* 23: 1842.

Coats, J. R. 1991. Pesticide degradation mechanisms and environmental activation. In "*Pesticide transformation products: Fate and significance in the environement*" (Eds. Somasundaram, L. and Coats, J. R.). pp. 10–31. ACS Symposium Series 459. American Chemical Society, Washington DC.

Code of Federal Regulations (CFR), USA. 1996. Protection of environment. Chapter 1. Environmental Protection Agency, Title 40, Pesticide Tolerance / Commodity / Chemical Index, Federal Register, Washington DC., USA.

Cooley, D. R. and Manning, W. J. 1995. Estimating the risks and benefits of pesticides: Considering the agroecosystem and integrated pest management in the use of EBDC fungicides on apples. *Environmental Pollution.* 88: 315– 320.

Cremlyn, R. 1978. *Pesticides.* Preparation and mode of action. Wiley, NY.

Cruickshank, P. A. and Jarrow, H. C. 1973. Ethylenethiourea degradation. *J. Agric. Food Chem.* 21: 333–335.

Downing, L. D. 1989. *Processed apple products.* Van Nostrand Reinhold, New York.

DuPont de Nemours and Company. 1983. Technical data sheet for mancozeb. Biochemical Department. Wilmington, DE: DuPont.

Dychdala, G. R. 1991. Chlorine and chlorine compounds. In "*Disinfection, Sterilization, and Preservation*". pp. 157–182, Lea & Febiger. Philadelphia, PA.

Easton, T. 1951. The uses of ozone. Austral. *J. Dairy Technol.* 4: 142–143.

Ecobichon, D. J. 1994. Carbamic acid ester insecticides. In "*Pesticides and neurological disease*". 2nd Ed. pp. 171–249. CRC Press. FL.

Ecobichon, D. J. 1996. Toxic effects of pesticides. In "*Casarett & Doull's Toxicology: The basic science of poisons*" pp. 676-679, McGraw–Hill Companies, Inc.

Edwards, R., Ferry, D. G. and Temple, W. A. 1991. Fungicides and related compounds. In "*Handbook of pesticide toxocology. Classes of pesticides*". Vol 3. pp. 1409–1470. NY.

El-Hadidi, M. F. 1993. Studies on pesticide residues in fresh and processed apple fruits under certain developed pest control programs. Ph.D. Dissertation. Department of Economic Entomology, Faculty of Agriculture, Cario University, Egypt.

EPA. 1989. Preliminary risk assessment. EPA. ENV. Sci. Div. Reg. 5 to 5173535598. 8–19

Egli, H. 1982. Storage stability of pesticide residues. *J. Agric. Food chem.* 30: 861–866.

Engst, R. and Schnaak, W. 1974. Residues of dithiocarbamate fungicides and their metabolites on plant foods. *Residue Reviews.* 52: 45–67.

Fahey, J. E., Nelson, P. E. and Gould, G. E. 1971. Removal of Azodrin residues from tomatoes by commercial preparative methods. *J. Agric. Food Chem.* 19: 81–82.

Fair, G. M., Morris, J. C., Chang, S. L., Weil, I. And Burden, R. P. 1948. Chlorine as a water disinfectant. *J. Am. Water Works. Assoc.* 40(10): 1051.

Farrow, R. P., Elkins, E. R., Rose, W. W., Lamb, F. C., Ralls, J. W. and Mercer, W. A. 1969. Canning operations that reduce insecticide levels by commercial and home preparation methods. *J. Agric. Food Chem.* 16: 65–71.

FDA. 1995. Beverages: Bottled water; final rule. Food and Drug Admin., Fed Reg. 60: 57075–57130.

Federal Register 1989. Office of the Federal Register, National Archives and Records Service, General Service Administration. Dec. 20

Fishbein, L. 1976. Environmental health aspects of fungicides dithiocarbamates. *J. Toxicol. Environ. Health.* 1: 713–716.

Fishbein, L. and Fawkes, J. 1965. Thin layer chromatography of metallic derivatives of ethylenebis(dithiocarbamic) acid and their degradation products. *J. Chromatogr.* 19: 364–369.

Foegeding, P. M. 1983. Bacterial spore resistance to chlorine compounds. *Food Technol.* 37 (11): 100–104.

Food Marketing Institute. 1992. Trends 92: Consumer attitudes and the supermarket. Washington, DC. Food Marketing Institute, 73.

Forney, C. F., Rij, R. E., Denis-Arrue, R. and Smilanick, J. L. 1991. Vapor phase hydrogen peroxide inhibits postharvest decay of table grapes. *Hort Science* 25: 1512–1514.

Fuhr, F. 1982. Fate of herbicide chemicals in the agricultural environment with particular emphasis on the application of nuclear techniques, in *Agrochemicals: Fate in Food and the Environment,* International Atomic Energy Agency, Vienna, pp 63–82.

Geisman, J. R. 1975. Reduction of pesticide residues in food crops by processing. *Residue Reviews* 54: 43–54.

Glaze, W. H. 1987. Drinking water treatment with ozone. *Environ. Sci. Technol.* 21(3): 224–230.

Gomaa, H. M. and Faust, S. D. 1974. Removal of organic pesticide from water to improve quality. In "*Pesticides in Soil and Water*", pp.413–450, Soil Science Society of America, Inc., Madison, WI.

Graham, D. M. 1997. Use of ozone for food processing. *Food Technol.* 51(6): 72–75.

Graham, S. L., Davis, K. J., Hansen, W. H. and Graham., C. H. 1975. Effects of prolonged ethylenethiourea ingestion on the thyroid of the rat. *Food Cosmetol. Toxicol.* 13: 493–499.

Hajslova, J. 1999. Pesticides. In "*Environmental contaminants in food*". pp. 215–271. CRC Press. FL.

Hallenbeck, W. H. and Cunningham-Burns, K. M. 1985. Pesticides and human health. Springer–Verlag. NY.

Han, J. C.–Y. 1977. "Stability of ^{14}C maneb residues in canned vegetables", as reported in response of E.I. duPont de Nemours and Co. to RPAR. Vol. II. Appendix 4.

Handbook of Chemistry and Physics, 56th Edition. 1975-76. p. D–141–143, CRC Press Inc., Cleveland OH.

Hayes, W. J. 1982. Pesticides studied in man. Williams & Wilkins, Baltimore.

Hayes, W. J. and Laws, E. R. 1990. *Handbook of Pesticide Toxicology,* Vol. 3, Classes of Pesticides. Academic Press, Inc., NY.

Hendrix, F. F. 1991. Removal of sooty blotch and flyspeck from apple fruit with a chlorine dip. *Plant Disease.* 75: 742–743.

Hewes, C. G. and Davison, R. R. 1971. Kinetics of ozone decomposition and reaction with organics in water. *J. Am. Inst. Chem. Engrs.* 71(1): 141.

Honnay, R. 1988. Process for improving the preservation of fresh vegetables and fruits. Europ. Patent 0 255 814.

Hotchkiss, J. H. 1992. Pesticide residue controls to ensure food safety. Critical reviews in food science and nutrition. 31: 191–203.

IARC Monograph. 1974. Etylenethiourea. 7: 153–159.

Jennings, R. 1996. Ozone water treatment to aid habitat of salmon. Contra Costa Times, Concord, Calif., Nov. 15.

Johnston, A. C., Hoseney, R. C. and Ghiaski, K. 1980. Chlorine treatment of cake flours. V. Oxidation of Starch. *Cereal Chem.* 57: 94–97.

Kakalikova, L., Ragala, P. and Rajuiakova, O. 1988. Ethylenethiourea in musts and wines from Vlasina Riesling grapes treated with mancozeb. Vinohrad (Bratislava) 26: 135–137.

Kearney, P. C., Muldoon, M. T., Somich, C. J., Ruth, J. M. and Voaden D. J. 1988. Biodegradation of ozonated atrazine as a wastewater disposal system. *J. Agric. Food Chem.* 36: 1301–1306.

Klapes, N. A. and Vesley, D. 1990. Vapor-phase hydrogen peroxide as a surface decontaminant and sterilant. *Appl. Environ. Microbial.* 56: 503–506.

Kobayashi, K., Maekawa, T., Imada, N. and Ohsima, Y. 1990. Studies on the relation between toxicity and metabolism of organophosphorus insecticides in shrimp larvae at different stages to fenitrothion , an organophosphorus insecticide. *Bull. Japan Soc. Scien. Fish.* 56: 489–496.

Kopperman, H. L., Kuehl, D.W. and Glass, G. E. 1978. Chlorinated compounds found in waste treatment effluents and their capacity to bioaccumulate. In "*Water chlorination : Environmental Impact and Health Effects,*" Ann Arbor Sci. Publ.,Inc., Ann Arbor, Mich.1: 311–315.

Kotula, A. W., Lusby, W. R., Crouse, J. D. and deVries, B. 1974. Beef carcass washing to reduce bacterial contamination. *J. Anim. Sci.* 39: 674–679.

Kramer, W. 1983. Fungicides and bacteriocides. In "*Chemistry of pesticides*", pp. 227–321. Wiley, NY.

Lane, J. P. 1974. Sanitation recommendations for fresh and frozen fish plants. Fishery Facts-Natl. Ocean. And Atmos. Admin. Seattle, Wash.

Latshaw, C. L. 1994. Chlorine dioxide: effective, broadspectrum biocide for white-water systems. *Tappi Journal*, 78: 163–166.

Laubusch, E. J. 1962. Water chlorination. In "*Chlorine: Its manufacture, properties and uses.*" pp. 457-484. Ed. J. S. Sconce, Reinhold Publishing Corporation, New York.

Lentza–Rizos, C. 1990. Ethylenethiourea (ETU) in relation to use of ethylene bisdithiocarbamate (EBDC) fungicides. *Reviews of Environmental Contamination and Toxicology,* 115: 1–37.

Lillard, H. S. 1979. Levels of chlorine and chlorine dioxide of equivalent bacterial effect in poultry processing water. *J. Food Sci.*, 44: 1594–1597.

Lillard, H. S. 1980. Effect on broiler carcasses and water of treating chiller water with chlorine or chlorine dioxide. *Poult. Sci.* 59: 1761–1766.

Manhart, W. 1995. *Apples for the Twenty-First Century.* Published by the North American Tree company, Portland, OR.

Marshall, W.D. 1977. Thermal decomposition of ethylenebisdithio–carbamate fungicides to ethylenethiourea in aqueous media. *J. Agric. Food Chem.* 25: 357–361.

Marshall, W. D. 1979. Oxidative degradation of ethylenethiourea (ETU) and ETU progenitors by hydrogen peroxide and hypochlorite. *J. Agric. Food Chem.* 27: 295–299.

Marshall, W. D. 1982. Preprocessing oxidative washes with alkaline hypochlorite to remove ethyenebis(dithiocarbamate) fungicide residues from tomatoes and green beans. *J. Agric. Food Chem.* 30: 649–652.

Marshall. W. D. and Jarvis, W. R. 1979. Procedures for the removal of field residues of ethylenebis(dithiocarbamate) (EBDC) fungicide and ethylenethiourea (ETU) from tomatoes prior to processing into juice. *J. Agric. Food Chem.* 27: 766–769.

Masschelein, W. H. 1984. Methods for controlling chlorine dioxide operation. Part 2. *J. Awwa.* 3: 80–82.

McConnell, A. L. 1991. Evaluation of wash treatments for the imporvement of quality and shelf life of fresh mushrooms (Agricus bisporus). M.S. Thesis, Dept. of Food Science, Pennsylvania State Univ., University Park.

Meister, R. T. 1992. Farm Chemicals Handbook '92. Meister Publishing Company, Willoughby, OH.

Meneguz, A. and Michalek, H. 1987. Effect of zineb and its metabolite ethylenethiourea, on hepatic microsomal systems in rats and mice. *Bull Environ Contam Toxicol.* 38: 862–867.

Merwin, I. A., Brown, S. K., Rosenberger, D. A., Cooley, D. R. and Berkett, L. 1994. Scab-resistant apples for the northeastern United States: New prospects and old problems. *Plant Dis.*, 78: 4–10.

Miller. G. W., Rice, R. G., Robson, C. M., Scullin, R. L., Kuhn, W. and Wolf, H. 1978. Chlorine Dioxide. In, "*An assessment of ozone and chlorine dioxide technologies for treatment of municipal water supplies.*" pp. 179–224. Municipal Environmental Research Laboratory. US EPA. Cincinnati, OH.

Moffat, C. F. and Whittle, K. J. 1999. In "*Environmental Contaminants in Food*". Chapter 7. Pesticide. CRC Press.

Moody, M. W. 1976. Seafood plant sanitation. LSU Coop. Ext. Serv. Bull. Louisiana State NIV., Baton Rouge, LA.

Morgan, D. P. 1982. Recognition and management of pesticide poisonings. Third edition. Washington, DC: U.S. Environmental Protection Agency. U. S. Government Printing Office.

Morris, J. C. 1966. "The acid ionization constant of HOCl from 5 to 35C", *J. Phys. Chem.* 70: 3798–3802.

Nash, R. G. 1976. Uptake of ethylenebis(dithiocarbamate) fungicides and ethylenethiourea by soybeans. *J. Agric. Food Chem.* 24: 596–601.

Newsome, W. H. 1972. Determination of ethylenethiourea residues in apples. *J. Agric. Food Chem.* 20: 967–969.

Newsome, W. H. 1976. Residues of four ethylenebis(dithiocarbamates) and their decomposition products on field-sprayed tomatoes. *J. Agric. Food Chem.* 24: 999–1001.

Newsome, W. H. and Laver, G. W. 1973. *Bull. Environ. Contam. Toxicol.* 10: 151–154.

Newsome, W. H., Shields, J. B. and Villeneuve, D. C. 1975. Residues of maneb, ethylenethiouram monosulfide, ethylenethiourea and ethylenedizmine on beans and tomatoes field treated with maneb. *J. Agric. Food Chem.* 23: 756–758.

Occupational Health Services (OHS), Inc. 1991. MSDS for Mancozeb. OHS Inc., Secaucus, NJ.

Ong, K. C., Cash, J. N., Zabik, M. J. Siddiq, M. and Jones, A. L. 1996. Chlorine and ozone washes for pesticide removal from apples and processed apple sauce. *Food Chemistry.* 55(2): 153–160.

Ott, S. L., Misra, S. and Huang, C. L. 1991. Improving supermarket sales of organic produce. In Food Review; Organic food and the consumer. U.S. Department of Agriculture Economic Research Service. 14: 6–8.

Phillips, W. F., Grady, M. D. and Freudenthal, R. 1977. "Effect of food processing on ethylenebisdithiocarbamate fungicides and ethylenethiourea report" prepared for health effects research lab, U.S. EPA, National Technical Information Service, U.S. Department of Commerce PB–268653.

Racke, K. D. and Coats, J. R. 1990. *Enhanced Biodegradation of Pesticides in the Environment,* ACS Symposium Series 426, American Chemical Society, Washington, DC.

Ranken, M. D., Clewlow, G., Shrimpton, D. H. and Stevens, B. J. H. 1965. chlorination in poultry processing. *Br. Poult. Sci.* 6: 331–337.

Reagan, J. O., Acuff, G. R., Bulge, D. R., Buyck, N. J., Dickson, J. S., Kastner, C. Massden, J.L., Morgan, J. B., Nickelson, R., Smith, G. C. and Sofas, J. N. 1996. Trimming and washing of beef carcasses as a method

of improving the microbiological quality of meat. *J. Food Protect.* 59: 751–756.

Reina, L. D., Fleming, H. P. and Humphries, E. G. 1995. Microbial control of cucumber hydrocooling water with chlorine dioxide. *J. Food Protect.* 58: 541–546.

Rhodes, R. C. 1977. Studies with manganese [^{14}C] Ethylenebis (dithiocarbamate) [^{14}C]ETU) in plants, soil and water. *J. Agric. Food Chem.* 25: 528–533.

Richardson, S. D. 1998. Drinking water insinfection by-products. In "*The Encyclopedia of Environmental Analysis & Remediation*", ed. R. A. Meyers, Vol. 3. pp 1398–1421. John Wiley & Sons, NY

Richardson, S. D., Thruston, A. D., Jr., Collette, T. W., Patterson, K. S., Lykins, W. Jr., Majetich, G. and Zhang, Y. 1994. Multispectral identification of chlorine dioxide disinfection by–products in drinkingwater. *Environ. Sci. Technol.* 28: 592–599.

Rij, R. E. and Forney, C. F. 1995. Phytotoxicity of vapor phase hydrogen peroxide to Thompson seedless grapes and *Botrytis cinerea* spores. Crop Prot. 14: 131–135.

Ripley, B. D. and Cox, D. F. 1978. Residues of ethylenebis-(dithiocarbamate) and ethylenethiourea in treated tomatoes and in commercial tomato products. *J. Agric Food Chem* 26: 1137–1143.

Rohm and Haas Company. 1997. Material safety data sheet. 62804. Rohm and Haas Company. Philadelphia, PA.

Ronk, R. 1975. Status of ozone for water treatment and food processing under the Federal FD&C Act. In proc. Of 1st Intl. Symp. on ozone for water and waste water treatment. Intl. Ozone Assn., Stamford, Conn. 830–842.

Rose, D., Pearson, C. M., Zuker, M. and Roberts, J. R. 1980. Ethylenethiourea: Criteria for the assessment of its effects on man. National Research Council Canada : Ottawa, Ontario, Canada, Publ. No. 18469.

Ross, R. D. and Crosby, D. G. 1973. Photolysis of ethylenethiourea. *J. Agric. Food Chem.* 21: 335–337.

Ross, R. G., Wood, F. A. and Stark, R. 1978. Ethylenebis-dithiocarbamate and ethylenethiourea residues in apples and apple products following sprays of mancozeb and metiram. *Can J. Plant Sci.* 58: 601–604.

Sapers, G. M., Miller, R. L. and Simmons, G. 1995. Effects of hydrogen peroxide treatment on fresh-cut fruits and vegetables. Presented at Ann. Mtg., Inst of Food Technologists, Anaheim, Calif., June 3–7.

Sapers, G. M. and Simmons, G. F. 1998. Hydrogen peroxide disinfection of minimally processed fruits and vegetables. *Food Technol.* 52(2): 48–52.

Sarig, P., Zahavi, T., Zutkhi, Y., Yannai, S., Lisher, N. and Ben-Arie, R. 1996. Ozone for control and postharvest decay of table grapes caused by *Rhizopus stolonifer. Physiol. Molec. Plant Pathol.* 48: 403–415.

Schubert, S., Shistar, T. and Feldman, J. 1996. Voices for pesticide reform: The case for safe practices and sound policy. Washington, D. C.

Seiler, J. P. 1973. Ethylenethiourea (ETU), a carcinogenic and mutagenic metabolite of ethylenebisdithiocarbamate. *Mut. Res.* 26: 189–191.

Shepard, T. 1989. Catalog of teratogenic agnets. Fifth edition. Baltimore, MD: The Johns Hopkins University Press.

Sherma, J. 1997. Current status of pesticide residue analysis. *J. AOAC international.* 80: 283–287.

Shukla, Y., Antony, S., Kumar, S. and Mehrotra, N. D. 1990. Carcinogenic activity of a carbamate fungicide, mancozeb on mouse skin. *Cancer Letters*, 53: 191–195.

Siler, M. D. 1998. The use of preharvest intervals, postharvest wash treatments, and processing in the removal of pesticides from apple fruit. M. S. Thesis. Department of Food Science, Michigan State University, MI.

Song, J., Fan, L. and Beaudry, R. M. 1998. Application of solid phase microextraction and gas chromatography/Time-of-flight mass spectrometry for rapid analysis of flavor volatiles in tomato and strawberry fruits. *J. Agric Food Chem.* 46: 3721–3726.

Song, J., Gardner, B., Holland, J. and Beaudry, R. 1997. Rapid analysis of volatile flavor compounds in horticultural produce using SPME and GC/time-of-flight mass spectrometry. *J. Agric. Food Chem.* 44: 2187–2193.

Standard Methods for Examination of Water and Wastewater. 1987. 17th Ed., pp. 162–165, 298–300, American Public Health Assoc., New York.

Stockinger, H., Heinzle, E. and Kut, O. M. 1994. Combination of ozonation and biological treatment of waste water containing biorefractory chloro– and nitroaromatic pollutants. In, "*Proceedings International Ozone Conference*". pp. 165–176, Zurich, Switzerland.

Tafuri, F. T., Businelli, M., Scarponi, L. and Giusguiani, P. L. 1970. Chlorocholine chloride residue in grapes and their fate in winemaking. *J. Agric. Food Chem.* 18: 869–871.

Teramoto, S., Moriya, M., Kato, K., Tezuka, H., Nakamura, S., Singu A. and Shirasu, Y. 1977. Mutagenicity testing on ethylenethiourea. *Mutat. Res.* 56: 121–129.

Teramoto, S., Saito, R. and Shirasu, Y. 1980. Teratogenic effects of combined administration of ethylenethiourea and nitrate in mice. *Teratology* 21: 71–78.

Uesugi, Y. 1998. Fungicide classes: Chemistry, uses and mode of action. In "*Fungicidal Activity: Chemical and biological approaches to plant protection.*" pp. 23–56. Ed. Hutson, D and Miyamoto, J., John Wiley & Sons Ltd., England.

Ulland, B. M., Weisburger, J. H. Weisburger, E. K., Rice, J. M. and Cypher, R. 1972. Brief communication: thyroid cancer in rats from ethylene thiourea intake. *J. Natl. Cancer Inst.* 49: 583–584.

Uno, M., Okada, T., Onji, Y., Matubara, S. and Veda, E. 1978. H. *Food Hyg. Soc. Jpn.* 19: 397.

U. S. Environmental Protection Agency. 1982. Ethylene bisdithiocarbamate pesticides. Final resolution of rebuttable presumption against registration. Decision document. Office of Pesticide Programs.

U. S. Environmental Protection Agency. 1986. Task 2: Environmental fate and exposure assessment. Final report. June 10.

U. S. Environmental Protection Agency. 1987. Pesticide fact sheet: Mancozeb, Registration Standard. Office of Pesticides and Toxic Substances. Office of Pesticide Programs, Washington, DC.

U. S. Environmental Protection Agency. 1992. Ethylene bidithiocarbamates (EBDCs); Notice of intent to cancel and conclusion of Special Review. Federal Register 57(41): 7434–7530. US GAO, Washington, DC.

Van der Poll, J. M., Versluis-de Haan, G. G. and de Wilde, O. 1993. Determination of ethylenethioure`a in water samples by gas chromatography with alkali flame ionization detection and mass spectrometric confirmation. *J. Chroma.* 643: 163–168.

Von Stryk F. G. and Jarvis, W. R. 1978. Residues of mancozeb, maneb and ethylenethiourea in fungicide-treated field and green–house tomatoes. *Can. J. Plant Sci.* 58: 623–628.

Wang, J. and Toledo, R. T. 1986. Sporicidal properties of mixtures of hydrogen peroxide vapor and hot air. *Food Technol.* 40(12): 60–67.

Watts, R. R., Stoherr, R. W. and Onley, J. H. 1974. Effect of cooking on ethylenebisdithiocarbamate degradation to ethylenethiourea. *Bull. Environ. Contam. Toxicol.* 12 (2): 244–226.

Way, R. D. and McLellan, M. R. 1989. Apple cultivars for processing. *Processed Apple products*, Van Nostrand Reinhold, New York, NY. pp. 1–29.

Wei, C. I., Cook, D. L. and kirk, J. R. 1985. Use of chlorine compounds in the food industry. *Food Tech.* 39(1): 107–115.

Windholz, M., Budavari, S., Blumetti, R. F. and Otterbein, E. S. 1983. The Merck Index, 10th ed. Rahway, NJ: Merck and Co., Inc.

Yefchak, G. E. 1990. Improvements to resolving power in time-of-flight mass spectrometry. Ph. D. Dissertation. Department of Chemistry, Michigan State University, MI.

Yip, G., Onley, J. H. and Howard, S. F. 1971. Residues of maneb and ethylenethiourea on field sprayed lettuce and kale. *J. Assoc. Office Anal. Chem.* 54: 1373–1375.

Zepp, R. G. 1991. Photochemical fate of agrochemicals in natural waters. In "*Pesticide chemistry: Advances in international research, development and legislation*" (ed. Frehse, H.). pp. 329–346. VCH, Weinheim.